CHAPTER I

CHAKRAS
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CHAKSINE

Historical: Cassia absus Linn. is an indigenous medicinal plant which belongs to the natural order Leguminosae. It is an annual plant, 1-2 ft. high and is found throughout India, Ceylon, other parts of tropical Asia, Australia and Africa. Its flat, oblong seeds are dark and contain a kernel used in the treatment of Ophthalmia, skin affections and as a cathartic. In North India the seeds also find considerable use for blood purification, bronchitic pains, leucoderma etc.

The presence of an alkaloidal principle in Cassia absus was first reported by Dymock. Siddiqui and Ahamad, however, were the first to isolate a water-soluble base in a total yield of about 1.3 per cent. This was named Chaksine after the vernacular name "Chaksu" for Cassia absus.

First experiments on the pharmacological action of chaksine were also reported by Siddiqui and Ahamad, who showed that dilute aqueous solutions of
chaksine sulphate on frogs acted as a general depressant of heart, respiration and nerves. The lethal dose in the case of frogs was of the order of 0.1 gm. per kilogram. More recently De et al.\(^4\) have carried out detailed pharmacological examination of chaksine and found that the alkaloid possesses "curariform like activity".

Siddiqui and Ahamad\(^3\) described chaksine as a quarternary base and prepared several of its crystalline salts. The free base could not be obtained in a pure condition on account of the strong avidity which it exhibited for carbon dioxide from the air. These authors also assigned a molecular formula \(C_{12}H_{21}O_2N_3\) to the alkaloid, but their conclusions were later challenged by Ray et al.\(^5\), who showed that the formula \(C_{11}H_{21}O_3N_3\) represented the molecule with greater fidelity.

More than half a dozen papers had been published by different groups when the work on chaksine was undertaken by us in the year 1955. Most of these workers concerned themselves with the demonstration of the nature of the functional groups and also attempted several hydrolytic and oxidative degradations. However, it had not been possible till then to establish the structure or provide any indication of the type of system present in chaksine.
As a result of the experiments carried out by us, we are now in a position to assign a tentative structure to the alkaloid, which we consider to be a satisfactory expression for explaining all the known reactions of the natural product. The details regarding this formulation are discussed in subsequent pages.

Chemical examination of Cassia-absus: The earliest work on the composition of the seed was first reported by Dymock, who carried out the estimation of the moisture content, the ash percentage, the alkaloidal principle, etc. Ahamad had also reported on the composition of the fatty material present in the seeds. Recently Gupta et al. have carried out a more thorough chemical examination of the oils. The procedure recommended by Siddiqui and Ahamad for the isolation of chaksine was found by us to be quite suitable. The seeds were crushed and ground to a fine yellow powder. The broken husks were removed by passing through a sieve. Repeated extraction with 0.3 per cent methanolic hydrogen chloride in the cold, followed by basification with ammonia and reacidification with acetic acid and removal of the solvent under vacuum yielded a yellow syrup containing the chaksine salt. This was freed from the fatty material
by extraction with ether. Treatment with a saturated solution of ammonium sulphate or potassium iodide resulted in the precipitation of chaksine sulphate or chaksine iodide. In this way it was possible to isolate the crude alkaloid in 1.5-1.9 per cent yield. Fresh seeds gave a better yield of the alkaloid.

Besides chaksine, the isolation of another isomeric quarternary base iso-chaksine was also claimed by Siddiqui and Ahamad. It was reported that the separation of the two alkaloids could be effected by taking advantage of the difference in the solubilities of their sulphates and iodides in water and alcohol respectively - the salts derived from iso-chaksine being more easily soluble. During our investigations we looked for iso-chaksine but were unable to isolate the base or any of its salts. Neither could we find any evidence pointing towards the presence of iso-chaksine. Ray et al. have also recorded a similar experience.

A careful examination of the mother liquors led, however, to the isolation of two more crystalline products which were found to be void of any nitrogen content. The aqueous residues which had been freed of the precipitated chaksine sulphate were allowed to concentrate at room temperature for several days, when
a small amount of the colourless mass had collected at
the bottom. This, after several crystallizations from
water, had a melting point 205°. Similarly, the com-
bined ether extracts containing the fatty residues
were concentrated to remove all ether and the residual
oily mass was left in a refrigerator for several days.
A small amount of the solid material had again collec-
ted. After crystallization from boiling ethanol into
a micro crystalline powder; this had a melting point
260°. We have not carried out any work which might
help in identifying these two products.

Properties of chaksine: (A review of the earlier de-
gradative work): Siddiqui and Ahamad\(^3\) described
chaksine as an optically active, strong quarternary
base, freely soluble in water. Their attempts at iso-
lation of the free base by decomposition of chaksine
iodide with silver oxide resulted in failure because,
during the course of isolation, the free base had conver-
ted into chaksine carbonate after absorption of carbon
dioxide from the air. These authors and later on Ray
et al.\(^5\) were, however, able to prepare several highly
crystalline salts of chaksine, like chloride, bromide,
nitrate, acetate, oxalate, carbonate, succinate, tartrate,
etc. These were made either by the decomposition of the
carbonate with respective acids or through double decom-
position, etc. Chaksine also formed a crystalline
picrate and a double salt with platinic chloride. On
treatment with benzyol chloride in the presence of a mild
alkali, the isolation of two different benzyol deriv-
atives was reported by Siddiqui et al.\textsuperscript{7}. Possible for-
mulation for these derivatives would be discussed at a
later stage.

With sodium hydroxide and barium hydroxide
the evolution of ammonia was first reported by Ray et al.\textsuperscript{5}
though no crystalline products were isolated. Treatment
of chaksine nitrate with concentrated sulphuric acid was
reported by this group to result in the loss of a mole-
cule of water leading to the formation of nitrochaksine.
In a subsequent publication Ray et al.\textsuperscript{8}, however, claimed
extensive degradation of chaksine nitrate on treatment
with concentrated sulphuric acid leading to the formation
of three different products. The exact significance of
these reports is also discussed in subsequent pages.

Ray and coworkers\textsuperscript{5} also carried out an oxi-
dation of chaksine sulphate with hydrogen peroxide and
claimed the isolation of formaldehyde from the reaction.
We have not been able to repeat this observation. Simi-
larly, oxidation of chaksine with potassium permanganate
was also reported\textsuperscript{5,8} by these workers to yield oxalic acid,
adipic acid and two other acids isolated in the form of ethyl esters, $\text{C}_{10} \text{H}_{18} \text{O}_4$ and $\text{C}_7 \text{H}_{14} \text{O}_3$. We were again unable to repeat these observations except to confirm the formation of oxalic acid during the course of this oxidation. These workers had also studied the reaction of chaksine with one mole and large excess of nitrous acid. The formation of chaksine nitrite along with two other products $\text{C}_9 \text{H}_{13} \text{N}_3 \text{O}_4$ and $\text{C}_8 \text{H}_{13} \text{N}_3 \text{O}_4$ was reported. We have found that chaksine is recovered completely unchanged after interaction with nitrous acid, the only isolable compound being chaksine nitrate.

Siddiqui and coworkers$^9,10$ more recently isolated phthalic acid and para isopropylbenzoic acid from the pyrolytic degradation products of chaksine iodide. They degraded chaksine salt with copper powder above $300^\circ$ in nitrogen atmosphere. The strong caraway-smelling product thus obtained was refluxed for twelve hours with twenty per cent methanolic caustic potash to yield the acids.

**Molecular formula of chaksine:** Siddiqui and Ahamad$^3$ on the basis of the elemental analysis of chaksine bicarbonate assigned a molecular formula $\text{C}_{12} \text{H}_{21} \text{O}_2 \text{N}_3$ to chaksine. Chaksine bicarbonate was itself assigned the molecular
formula $\text{C}_{12}\text{H}_{20}\text{ON}_3\text{HCO}_3 \ 1/2 \text{H}_2\text{O}$, which was dried under vacuum at $100^\circ$ before analysis. Ray et al.\(^5\) objected to the assumption that the loss in weight under vacuum at $100^\circ$ was solely due to half a molecule of water, but considered that the bicarbonate of an organic base might also lose carbon dioxide under these conditions. Furthermore, there was considerable discrepancy in the reported values of the iodine in chaksine iodide by Siddiqui et al. Ray and coworkers\(^1\) preferred the formula $\text{C}_{11}\text{H}_{21}\text{O}_3\text{N}_3$ for the chaksine base and this received support from the analysis of the sulphate, nitrate, chloride and bromide of chaksine. This new formula was not accepted by Siddiqui et al.\(^1\) who maintained that the analysis of chaksine sulphate (melting point $317^\circ$) was not reliable on account of the high melting point and poor combustible nature, and that the other salts had not been carefully dried to constant weight under vacuum before analysis. Lalla and Gupta\(^1\) undertook this investigation and prepared several salts in a high state of purity and dried the samples thoroughly before analysis. Their results strongly favour the $\text{C}_{11}\text{H}_{21}\text{O}_3\text{N}_3$ formula for chaksine. This controversy was finally resolved in favour of the latter formulation from the analysis of oxygen present in chaksine hydrochloride carried out by us\(^1\). An analysis
of the platinum double salt obtained from chaksine hydrochloride also indicated molecular weight corresponding to $C_{11}H_{21}O_3N_3$. Zeisel and Meyer's estimations indicated the absence of any alkoxy or N-alkyl groups. Kuhn Roth estimation by Ray showed the presence of one C-methyl grouping.

The infrared spectrum of chaksine: When studied as a nujol mull chaksine iodide showed two strong bands at 5.81 $\mu$ and 5.98 $\mu$ in the double bond region, besides absorption bands at 3.0 $\mu$ and 7.24 $\mu$. In our first communication, we had also reported that when chaksine iodide was studied as a nujol mull in the infrared at somewhat higher concentration, a sharp band of medium intensity was also observed at 4.30 $\mu$. At a later stage when we had occasion to study the infrared of chaksine hydrochloride in the solid form in potassium bromide disc, this band was, however, found to be absent. Careful re-investigation led to the conclusion that the 4.30 $\mu$ band in chaksine iodide was really due to the presence of a small amount of ammonium iodide which had been used for the precipitation of chaksine iodide. When potassium iodide was used in place of ammonium iodide, the 4.30 $\mu$ band disappeared. As is well known, all the ammonium salts
CHNKSINE IODIDE IN KBr

WAVELENGTH MICRONS

NITROCHNKSINE IN CHLOROFORM

WAVELENGTH MICRONS
are expected to exhibit this absorption around the 4.30 region. The earlier observations, however, led to considerable confusion in the interpretation of the results of the infrared analysis. This is discussed in greater detail at a later stage. The 5.81 µ indicated the presence of a carbonyl group, an ester grouping or a lactone ring (larger than six membered – which in the infrared would behave like an ester). Similarly the 5.98 µ could arise from the presence of an enol ther, an amide or \(-\text{C} = \text{N}\)- function present in chaksine molecule. The 7.24 µ band was a further confirmation of the presence of a C-methyl group. The absorption at 3.0 µ indicated the presence of an NH or OH grouping.

Ultraviolet spectrum of chaksine: Chaksine does not show any absorption in the ultraviolet region, except for an inflection of low intensity at 271 mµ. This would indicate the absence of any conjugated system in chaksine and points to the more or less saturated nature of the molecule.

The functions of the nitrogen atoms: Siddiqui et al.\(^3,7\) and Ray et al.\(^5,8\) had always considered that one nitrogen atom of the chaksine molecule was quarternary in nature. This implied the presence of a quarternary ammonium nitrogen
attached to four carbon atoms or a quarternary immonium group
\[
\text{grouping } \: >C=\text{N} - \text{C} + \text{. This formulation was based mostly on the observation that the free chaksine base could not be isolated on account of its great solubility in aqueous solutions and its great avidity for carbon dioxide. Siddiqui}^7 \text{ also demonstrated that a second nitrogen atom was also feebly basic, because he could isolate the disulphate of chaksine in alcoholic solution. This disulphate was extremely susceptible to hydrolysis. We have been able to confirm this observation. The evolution of ammonia by treatment of chaksine with sodium hydroxide solution or baryta solution was recorded by all workers in the field, though no products were isolated and no conclusions were drawn. In our first communication, where we had accepted the findings of earlier workers about the quarternary nature of the nitrogen atom, we recorded that all the three nitrogen atoms were lost as ammonia on fusion with potassium hydroxide at 350°. This assumption derives strength from the experimental observations made by us that chaksine chloride was unaffected by treatment with diazomethane. Had it been an acid salt, the formation of the free base could be expected. In the earlier stages we preferred the quarternary ammonium}
to the quarternary immonium nature of the nitrogen atom. This preference was based on two observations: (i) the presence of a band of medium intensity at 4.30 µ in the infrared spectrum of chaksine iodide and (ii) the failure of chaksine iodide to undergo reduction with sodium borohydride, thus revealing the absence of $\text{C} \equiv \text{N}$. The lack of absorption in the triple bond region (page 10) 4.7 µ to 5.3 µ was further quoted by us to strengthen our supposition about the absence of a quarternary immonium nitrogen atom. At that time we had proposed that the 5.98 µ band could not have been due to $\text{C} \equiv \text{N}$ but was caused by the presence of an amide linkage in chaksine. As it turned out later, all these conclusions were ambiguous. We have already reported that chaksine hydrochloride does not exhibit any absorption band at 4.30 µ and that the presence of this band in chaksine iodide was really due to the impurity of ammonium iodide which had been used for the precipitation of chaksine iodide - the band having disappeared when potassium iodide was used as the precipitating agent.

At a later stage we found that chaksine base could actually be thrown out from an aqueous solution in the form of a semisolid mass by treatment with excess
sodium hydroxide or even ammonia solution. We were also able to isolate the base by repeated extraction with large quantities of purified ethyl acetate. The extract was dried thoroughly over anhydrous magnesium sulphate. Removal of the solvent yielded chaksine base as an amorphous powder which has resisted all attempts at crystallization so far. That no rearrangement had taken place during the decomposition of the salt was shown by the readiness with which the base formed the sulphate or the iodide in quantitative yield. The free base was found to be quite soluble in methylene chloride, though its solubility in chloroform was much less. The infrared spectrum of the crude base was similar to that of its hydrochloride in the relevant regions and all the four bands at 3.0, 5.81, 5.98 and 7.24 were present. Unfortunately it was not possible to prepare a crystalline sample for analysis. The free base could be stored indefinitely in a tightly stoppered bottle. The isolation of the free base and a study of its properties raised the doubt whether chaksine was really a quarternary base at all.

At this stage our attention was diverted to the reaction of chaksine nitrate with concentrated sulphuric acid. This reaction was first reported by Ray et al. in the year 1940 and later on studied by Ray and
Cuba\textsuperscript{8} in the year 1956 again in greater detail. These authors had reported extensive degradation of chaksine nitrate on treatment with concentrated sulphuric acid. Two different products were claimed to have been isolated. The major product obtained as a sulphate $(\text{C}_{11}\text{H}_{17}\text{N}_{4}\text{O})\text{SO}_4$ by diluting the reaction mixture, was reported to contain a carboxyl group. The second product was reported to be a base $\text{C}_8\text{H}_{13}\text{N}_3\text{O}$ and was obtained by neutralising the mother liquor. We\textsuperscript{16} have now found that the reaction leads to a simple nitration and results in the formation of nitrochaksine sulphate $(\text{C}_{11}\text{H}_{19}\text{N}_3\text{O}_2\text{NO}_2)\text{SO}_4$ melting point 170\textdegree C. as the sole product. The same results are obtained when chaksine sulphate is treated with a mixture of sulphuric and nitric acids. The absence of a carboxyl group was shown by its insolubility in the cold, in an aqueous solution of sodium bicarbonate or sodium carbonate. The free base nitrochaksine $\text{C}_{11}\text{H}_{18}\text{N}_3\text{O}_2\text{NO}_2$ obtained from the corresponding sulphate by neutralization with sodium bicarbonate and after crystallization from ethanol-chloroform mixture had a melting point 171\textdegree C and could be reconverted to the sulphate. Both the sulphate and the free base gave satisfactory analytical results. Similarly, the free base could be converted to its oxalate $\text{C}_{11}\text{H}_{19}\text{N}_3\text{O}_2\text{NO}_2\text{CO}^-$ which also gave satisfactory
values for the elemental analysis. The isolation and identification of the free nitrochaksine as $C_{11}H_{18}N_3O_3$ showed clearly that nitrochaksine and in its turn chaksine itself were not quarternary bases, otherwise the analytical values would correspond to the structure $C_{11}H_{20}N_3O_3NO_2$.

This nitration reaction is strongly suggestive of the well known conversion of guanidine nitrate $\rightarrow$ nitroguanidine by treatment of the former with sulphuric acid and indicates the presence of a guanidino function in the chaksine molecule. We have just referred to the fact that sodium borohydride was without action in chaksine. It is well known$^{17}$ that in general, the $C=\text{N}$ function in guanidines, amidines, hydrazidines is quite resistent to the action of lithium aluminium hydride and sodium borohydride and no reduction is observed in such cases. Thus the strong absorption band at $5.98 \mu$ to which reference has been made could be assigned to the $C=\text{N}$ portion of the guanidino function present in chaksine. Nakanishi et al. $^{18}$ have reported a value of $5.95 \mu$ for the alkaloid Roseonine (I) which has been shown to contain a guanidine function. In the ultraviolet chaksine

![Diagram of the structure of Roseonine (I) with a guanidino function.](image)
exhibited only weak absorption, but the low intensity inflection reported at 271 m\(\mu\) could be compared to the ultraviolet spectrum of the guanidine hydrochloride (\(\lambda_{\text{max}}; 265 \text{ m}\mu, \log e 1.26\)). These conclusions receive further support from the infrared spectrum of nitrochaksine (page 10) which shows the expected additional strong band at 6.60\(\mu\) and at 7.78\(\mu\) arising from the presence of a N - NO\(_2\) grouping. Patterson has given these values for several nitroguanidines at 6.23\(\mu\) and 7.6\(\mu\). In the ultraviolet the behaviour of nitrochaksine and nitroguanidine are also comparable. Thus the former exhibits \(\lambda_{\text{max}}\) at 275 m\(\mu\), while the latter has been reported by Jones to show \(\lambda_{\text{max}}\) at 269 m\(\mu\).

The behaviour of nitrochaksine and nitroguanidine towards alkali are again completely analogous. The former dissolves in the cold in a dilute solution of sodium hydroxide or on warming at 60\(^{\circ}\) with sodium carbonate solution. From the solution, after acidification, a solid acidic substance could be precipitated, which was found to be soluble in cold aqueous sodium bicarbonate. It has not been possible to crystallize the compound as it undergoes extensive degradation on boiling in different solvents. Nitroguanidines are also known to undergo hydrolysis on treatment with alkali and to yield
acidic substances. Further, when chaksine sulphate was
heated with dilute hydrochloric acid, carbon dioxide was
liberated. The resultant product, an urea, was no longer
basic and formed no picrate or platinum salt. It is thus
quite evident that all the three nitrogen atoms of chaksine
are successfully accounted for from the presence of a
guanidine function in chaksine. We have also reported that
chaksine hydrochloride did not show any absorption in the
triple bond region (4.7 μ - 5.3 μ ) and at an earlier
stage we had used this evidence implying the absence of a
N— function in chaksine. Witkop22 had shown the
+ presence of absorption bands in this region in all quarter-
ary immonium compounds. It seems important to point out
at this stage that guanidine hydrochloride itself did not
exhibit any absorption in this region. The absorption be-
+ behaviour of the \( \text{C}=\text{N}^- \) function in compounds studied by

Witkop cannot be compared with the system present in
guanidines. Reference has already been made to the for-
mation of two benzoates of chaksine by Siddiqui7 on treat-
ment with benzoyl chloride in the presence of mild alkali.
We have found that one of these benzoates (melting point
273°C) is a simple dibenzoate of chaksine and not a benzoyl
benzoate of chaksine as considered by Siddiqui et al. From
this it also follows that the nomenclature of the various
chaksine salts like "chaksine chloride", or "chaksine bromide", etc. should be changed to the more appropriate terminology of chaksine hydrochloride and chaksine hydrobromide, etc. It is also clear that the free chaksine base has the molecular formula $\text{C}_{11}\text{H}_{19}\text{O}_2\text{N}_3$ and not $\text{C}_{11}\text{H}_{21}\text{O}_3\text{N}_3$ as assigned by the earlier workers.

The alkaline degradation of chaksine: We have reported above that when chaksine iodide was fused with excess potassium hydroxide and the temperature slowly raised to $350^\circ$, all the nitrogen was evolved as ammonia. Towards the end of the fusion when the temperature has reached $300^\circ$, hydrogen gas was liberated and was collected over water. The examination of the residue yielded useful information. It was strongly acidified with hydrochloric acid and exhaustively extracted with ethyl acetate. Removal of the solvent yielded a thick syrup which was found to be strongly acidic. This was submitted to distillation under vacuum, when a liquid boiling over a range of $30 - 40^\circ$ was obtained. A study of the infrared of this distilled material showed absorption bands at $5.80\mu$ (presumably due to the presence of carboxyl groups) and also at $5.45\mu$ and $5.75\mu$ which indicated the presence of an anhydride - presumably formed during the course of
distillation. Further examination revealed the distillate to be a mixture of two acids, which we named chaksininic acid and chakainic acid. Their structures are discussed below.

The structure of chaksininic acid: The distillate of acids as obtained above was treated with excess phosphorus pentachloride in anhydrous toluene. After removal of the solvent etc. and phosphoryl chloride, the residual acid chloride was treated with excess of para toludine. The mixture of toludides thus obtained was repeatedly crystallized from dilute alcohol when a sharp melting (162°) product was obtained. Analysis revealed an empirical formula $C_{11}H_{14}ON$. This toludide was submitted to alkaline hydrolysis in fifteen per cent aqueous sodium hydroxide in the hope of isolating the parent acid. After four hours the reaction was interrupted when a solid acid containing nitrogen in a crystalline form (melting point 103°) was obtained in moderate yield. Analysis and molecular weight determination (Rast's method) indicated a formula $C_{15}H_{21}NO_3$. Taking into consideration that there must be at least one carboxyl group and a para toludino grouping, it was possible to expand the formula to $CH_3C_6H_4NOC(C_6H_4)COOH$. From this it was at once obvious that chaksininic acid is a
saturated dicarboxylic acid corresponding to the formula $\text{C}_8\text{H}_{14}\text{O}_4$. At this stage we made an unsuccessful attempt at the separation of chaksininic acid by fractional distillation from the mixture obtained as described above. The methyl esters obtained from the mixed acids by reaction with diazomethane, however, were found to be more amenable to separation by fractional distillation through a good column. It was possible to resolve it into two ester fractions boiling points $72-74^\circ/0.5$ mm. and $110-112^\circ/0.5$ mm. The first fraction after hydrolysis yielded an optically inactive acid which was obtained as a solid (melting point 56-58$^\circ$) after distillation (boiling point $150-152^\circ/3$ mm.). Analysis and neutral equivalent determination showed the expected molecular formula $\text{C}_8\text{H}_{14}\text{O}_4$. When this acid was distilled with lime, $\alpha$-methyl cyclohexanone was obtained in good yield, whose identity was established through melting point and mixture melting point determinations of the 2:4-dinitro phenylhydrazones ($134-136^\circ$) and the semicarbazones ($192-194^\circ$). This indicated that chaksininic acid was $1$-methyl pimelic acid (IV). Final confirmation was forthcoming from its synthesis by the alkaline ring opening of 2-methyl-2$'$-carboethoxy cyclohexanone (III), which in turn was obtained by the alkylation of the sodium salt of
2-carboethoxy cyclohexanone (II) by methyl iodide.

\[
\begin{align*}
\text{II} & \quad \text{III} & \quad \text{IV} \\
\text{O} & \quad \text{COOC}_2\text{H}_5 & \quad \text{O} & \quad \text{CH}_3 & \quad \text{COOH} & \quad \text{COOH} \\
\text{1. NaH} & \quad \quad & \quad \quad & \quad \quad & \quad \quad & \quad \quad \\
\text{2. MeI} & \quad \quad & \quad \quad & \quad \quad & \quad \quad & \quad \quad \\
\end{align*}
\]

Thirty gms. of chaksine iodide yielded six gms. of 1-methyl pimelic acid; (the name Chaksininc acid becomes redundant in view of its identity with the known 1-methyl pimelic acid).

The isolation of chaksinic acid: The high boiling ester on similar alkaline hydrolysis and extraction etc. yielded a thick viscous liquid which after freezing in liquid air and trituration etc. solidified. This after crystallization had a melting point 145-147° and was also found to be optically inactive. Two of the carboxyl groups present in chaksinic acid were so disposed that an anhydride formation could occur when the acid was distilled under suitable conditions. The anhydride which was a viscous liquid still contained a carboxyl group as shown by its ready effervescence with bicarbonate and the presence of
a band at 5.8 µ in the infrared. The formation of the anhydride was made use of in effecting a complete chemical separation of the acids as obtained after fusion with alkali. The mixture of the two acids after distillation was heated at 160-170° to ensure complete anhydride formation of the chaksinic acid. After redistillation, the mixture was treated in the cold with excess diazomethane in anhydrous ether. This treatment converted methyl pimelic acid into its dimethyl ester, while the third carboxyl group of the chaksinic acid was also esterified. Anhydride ring was not opened up by diazomethane of its contact for too long a time was avoided. The mixture was treated with aqueous potassium carbonate which opened up the anhydride ring of the chaksinic ester, while dimethyl pimilate remained in the ether layer. Complete saponification of the aqueous part yielded pure chaksinic acid. Separation of the ester by fractionation was however found to be more suitable. Thirty grams of chaksine iodide yielded three grams of crystalline chaksinic acid.

The structure of chaksinic acid: Analyses of chaksinic acid, its methyl ester and toluide, molecular weight by Rast's method, neutral equivalent determination through a potentiometric titration, indicated chaksinic acid to be
a tricarboxylic acid with a molecular formula corresponding to \( C_{10}H_{16}O_6 \). In the infrared, both the acid and the ester showed only one absorption band at 5.80 \( \mu \) in the double bond region. It had no absorption in the ultraviolet and the acid was recovered unchanged on attempted hydrogenation in the presence of Adam's catalyst. All these observations indicated that chaksinic acid was a saturated tricarboxylic acid. Chaksinic acid was recovered completely unchanged after fusion with potassium hydroxide at 300\(^\circ\) - no 1-methyl pimelic acid was formed during the reaction. It thus appeared that both 1-methyl pimelic acid and chaksinic acid were formed from chaksine by independent routes, the former could not be considered as the end product of the alkali reaction on the latter. A Kuhn Roth estimation indicated the absence of C-methyl in chaksinic acid. An examination of the infrared of the anhydride (bands at 5.45 \( \mu \) and 5.65 \( \mu \)) failed to reveal the size of the anhydride ring i.e. whether it was derived from a succinic acid or a glutaric acid, as the value obtained was intermediate for those known for succinic anhydride\(^{23}\) (5.36 \( \mu \) and 5.61 \( \mu \)) and glutaric anhydrides\(^{24}\) (5.55 \( \mu \) and 5.68 \( \mu \)). Taking all these facts into consideration, our first choice regarding an expression for chaksinic acid fell on the structure (V). This expression
(or its equivalent if present in chaksine), had the additional desired feature in that two of the carboxyl groups were situated in 1:5 positions and thus permitted the reversal of a Michael condensation (as shown in structure V), possibly resulting in the ultimate formation of 1-methyl pimelic acid via the unsaturated acid (VI).

\[
\begin{align*}
\text{CH}_2 & \quad \text{CH}_2 & \quad \text{CH} & \quad (\text{CH}_2)_4\text{COOH} \\
& & & \\
\text{COOH} & & \text{COOH} \\
\end{align*}
\]

V

\[
\begin{align*}
\text{CH}_2 & \quad \text{CH}_2 & \quad \text{CH} & \quad (\text{CH}_2)_4\text{COOH} \rightarrow \text{CH}_2 & \quad \text{CH} & \quad (\text{CH}_2)_4\text{COOH} \\
& & & & & \\
\text{COOH} & & \text{COOH} & & \text{COOH} \\
\end{align*}
\]

V

VI

1:3:7 heptane tricarboxylic acid (V) was synthesised according to the following scheme: 2-carboethoxy cyclohexanone (II) was converted into the corresponding sodium salt by interaction with sodium hydride in toluene and subsequently reacted with ethyl-\(\beta\)-bromopropionate to yield the ester (VII). The same product could also
be obtained by the interaction of (II) with ethyl acrylate in the Michael manner. Ring opening by boiling sodium

\[ \text{COOC}_2\text{H}_5 \quad \text{H}_5\text{C}_2\text{OOC-CH}_2\text{-CH}_2\text{O} \]

\[ \text{1. NaH} \quad \begin{array}{c} \text{II} \\
\text{2. Br-CH}_2\text{-CH}_2\text{-COOC}_2\text{H}_5 \\
\text{VII} \end{array} \]

ethoxide in ethanol, followed by alkaline hydrolysis gave 1:3:7-heptane tricarboxylic acid (V) which was found to have a melting point 78\(^\circ\) and was different from chaksinic acid. It is interesting to point out that the infrared spectrum of methyl 1:3:7 heptane tricarboxylate bore a very strong resemblance to that of methyl chaksinate and strongly indicated the close similarity of the two compounds. We also synthesised the corresponding 1:2:7-heptane tricarboxylic acid (XI) and 1:4:7-heptane tricarboxylic acid (XIX), but again found them to be different from chaksinic acid. The former was synthesised starting from cycloheptanone (VIII), which on condensation with ethyl carbonate in the presence of sodium hydride yielded the corresponding \(\beta\) - keto ester (IX). This was condensed with ethyl bromoacetate and the ester (X) thus obtained was again submitted to the hydrolytic opening of the heptanone ring to yield
the desired 1:2:7 heptane tricarboxylic acid (XI). It had a melting point 76–77°.

![Chemical structure](image)

The same acid was also synthesised starting from ethyl pimelate half aldehyde (XII). After condensation with malonic acid followed by hydrolysis and decarboxylation, the unsaturated acid (XIII) thus obtained was converted to its ethyl ester (XIV). This added the elements of hydrogen cyanide on treatment with sodium cyanide in ethanol to yield the cyanoester (XV). Further refluxing with concentrated hydrochloric acid gave the desired acid (XI), somewhat contaminated with the unsaturated acid (XIII). Separation was quite wasteful.
1:4:7-heptane tricarboxylic acid (XIX) was synthesised starting from 2-carboethoxy cyclopentanone (XVI) which was converted to the corresponding sodium salt and then condensed with ethyl γ-bromocrotonate to yield (XVII). This was subjected to hydrogenation in the presence of palladium-strontium carbonate, when the saturated keto ester (XVIII) was obtained. This was subjected to hydrolytic ring opening to give 1:4:7-
heptane tricarboxylic acid (XXI) melting point 120–121°.

Our conclusions regarding the absence of a C-methyl group in chaksinic acid was mainly based on the negative results of a Kuhn Roth estimation carried on the acid. However, a careful reexamination of the infra-red spectra of chaksinic acid or its methyl ester indicated the presence of a band at 7.24 µ which would imply the presence of a C-methyl grouping. At this stage our attention was directed to the work of Prelog, Barton and Woodward\(^{26,27}\), who had similar experience in the case of cevine tetracarboxylic acid (XX) where also the Kuhn Roth estimation gave negative result. According to these authors in such cases infrared spectra is more reliable.
and diagnostic. This led us to the consideration that

\[
\text{HOOC (CH}_2\text{)}_2 \quad \text{C} \quad \text{COOH} \\
\text{HOOC} \quad \text{CH} \quad \text{CH}_2\text{COOH}
\]

chaksinic acid could be represented by structure (XXVI) or (XXIX). (XXVI) was synthesised starting from ortho-methyl cyclohexanone (XXI), which on oxidation with chromic acid gave \(\delta\)-acetyl-\(\alpha\)-valeric acid (XXII). The corresponding methyl ester (XXIII) was condensed with methyl cyanoacetate according to the conditions recommended by

\[
\begin{array}{c}
\text{CH}_3\text{CO(CH}_2\text{)}_4\text{COOH} \quad \xrightarrow{\text{CH}_3\text{OH}} \quad \text{CH}_3\text{CO(CH}_2\text{)}_4\text{COOCH}_3 \\
\text{XXII} \quad \xrightarrow{\text{NaCN}} \quad \text{XXIII}
\end{array}
\]
Cope\textsuperscript{28}, to yield the unsaturated cyanoester (XXIV) which added the elements of hydrogen cyanide on treatment with sodium cyanide in ethanol to yield the dicyanoester (XXV). This was refluxed with concentrated hydrochloric acid, when the desired acid (XXVI) was obtained. It had a melting point 117-118\textdegree{} and was different from chaksinic acid.

The only other alternative was, that chaksinic acid had the structure (XXIX). This was synthesised\textsuperscript{29} according to the following scheme and was found to be identical with chaksinic acid. 2-methyl-6-carboethoxy-
cyclohexanone (XXVII) was alkylated by ethyl bromoacetate in the presence of potassium tertiary butoxide to yield 2-methyl-6-carboethoxy-cyclohexanone-6'-ethyl acetate (XXVIII). Ring opening by means of sodium ethoxide in boiling absolute ethanol followed by alkaline hydrolysis resulted in the formation of 1:2:6-heptane tricarboxylic acid (XXIX). This had a melting point of 145-147° alone or on admixture with a sample of chaksinic acid. The overall yield in the three steps was above 50 per cent. The tritoludide obtained from either sources had a melting point 245° and no depression in the melting point was observed in the presence of each other. The infrared absorption spectra of the acids (page 32) were found to be completely identical. This synthesis establishes that chaksinic acid is 1:2:6-heptane tricarboxylic acid (XXIX).

The functions of oxygen in the chaksine molecule: We have already explained the functions of the three nitrogen atoms in the chaksine molecule as arising from the presence of a guanidino part. The presence of ten carbon atoms in chaksinic acid and the additional eleventh carbon atom of the guanidine residue also accounts for all the carbon atoms of chaksine. This leaves us only with the two remaining oxygen atoms to be accounted for.
We have already referred to the presence of a strong absorption band in the infrared spectrum of chaksine at 5.81 µ. This indicated the presence of any one of the three functional groups (i) an ester, (ii) an aldehyde or ketone or (iii) a lactone grouping. The presence of an ester is easily ruled out since all the carbon atoms have been accounted for and, secondly, no alcohol was formed during hydrolysis of chaksine. Similarly, chaksine does not give any reaction for any aldehyde or a ketone and does not yield any carbonyl derivatives. This leaves us with the only alternative - that chaksine must incorporate a lactone function in its molecule. The five- and six-membered lactones are known to absorb in the region 5.76 - 5.78 µ in the infrared, but a large-membered lactone (which could be expected to behave like an ester) might absorb at somewhat higher wave length. Thus, the 5.81 µ absorption band could very well be due to an eight- or nine-membered lactone ring. At this stage the behaviour of chaksine towards aqueous alkali may be recalled. The free chaksine base is first thrown out, but on warming at 60-70° for several minutes, complete dissolution takes place, even though no ammonia is liberated. On cooling, the solution remains clear and no chaksine base separates out. It was, however, not possible to isolate any crystalline products from this reaction. In another experiment,
while dilute baryta solution had been used for hydrolysis and approximately one molecule of ammonia had been liberated, barium salt of a carboxylic acid was obtained. This on decomposition with carbon dioxide yielded an acidic substance. On further chromatographic purification by passing over a column of activated alumina, we were able to obtain a crystalline product in one experiment, which unfortunately was lost during further purifications etc. We were unable to repeat the isolation of this crystalline acid, despite repeated attempts. These experiments, however, do lend support to the suggestion of the presence of a lactone function in chaksine molecule.

Ray's structure for chaksine: In the year 1956 Guha and Ray, after extensive degradation of chaksine by treatment with a mixture of nitric acid and sulphuric acid and also by interaction with large excess of nitrous acid, arrived at structure (XXX) for chaksine. They claimed support for this structure from the infrared study of chaksine and several of its derivatives along with its degradation by interaction with fuming nitric acid.

We have already commented on the nitration reaction and shown that Ray's conclusions were in error. Very recently, Kamal and Hahn have corroborated our
results on the nitration reaction of chaksine. Similarly, we have also investigated the reaction of chaksine with nitrous acid in detail. Guha and Ray studied the interaction of chaksine chloride with one equivalent and a large excess of nitrous acid and reported the isolation of chaksine nitrite and two other products, $\text{C}_9\text{H}_{16}\text{N}_2\text{O}_3$ and $\text{O}_4$. Chaksine was recovered by us completely unchanged after reaction with nitrous acid; chaksine nitrate was the sole product obtained from this reaction. An aqueous solution of chaksine hydrochloride was treated with an excess of nitrous acid at room temperature and allowed to stand for 3-4 hours. On cooling the reaction mixture, chaksine nitrate separated out which crystallized from aqueous alcohol in silky needles; melting point 213°C; undepressed on admixture with an authentic sample prepared
by treating chaksine iodide with silver nitrate. This identity was further confirmed by the formation of oxalate and sulphate whose melting points were found to be 295° (decomp.) and 305° (decomp.) respectively, alone or in admixture with authentic samples. The base \( C_9H_{16}N_2O_3 \), claimed to have been isolated from the filtrates on neutralization with ammonia, was found by us to be the free chaksine base. This was also apparent from the melting point of its oxalate 298° (decomp.) as reported by Guha and Ray. We have confirmed its identity through mixture melting point determination with an authentic sample. The compound, \( C_8H_{13}N_3O_4 \), melting point 210° (decomp.) also claimed to have been isolated by acidifying the ammoniacal solution with acetic acid followed by concentration, was found by us to be chaksine acetate. The melting point was also undepressed after admixture with an authentic sample (melting point 210-211° decomp.). The formation of chaksine nitrate is not difficult to understand when it is realized that nitrous acid can easily be converted to nitric acid in the presence of atmospheric oxygen.

The interpretation of the various infrared spectra described by these authors is inadequate and most of their other experimental work reported, cannot be reproduced by us. This work has no merit and does not
deserve any further consideration.

The structure of chaksine: Before attempting to write a satisfactory expression for chaksine, it would be desirable to put all the salient features regarding chaksine chemistry at one place. Thus we have in chaksine:

1) Molecular formula \( \text{C}_{11}\text{H}_{19}\text{O}_{2}\text{N}_3 \).

2) The presence of a guanidine function: \( -\text{NH} \rightarrow \text{C}=\text{NH} \).

3) The presence of a large-membered lactone function: \( -\text{CO} \cdots \text{O} \).

4) The formation of 1:2:6-heptane tricarboxylic acid, and 1-methyl pimelic acid along with the evolution of hydrogen and ammonia gases during the alkali fusion of chaksine.

5) The isolation of p-isopropyl benzoic acid from the thermal degradation of chaksine in the presence of copper by Siddiqui et al.

The molecular formula \( \text{C}_{11}\text{H}_{19}\text{O}_{2}\text{N}_3 \) for chaksine indicate the presence of four functions in the molecule. Three of these are accounted for from the double bond of the guanidine part, the double bond of the carbonyl of the lactone function and the lactone ring. This leads us to the further conclusion that there must be present a second
ring in the chaksine molecule. All these factors are uniquely accounted for in the structure (XXXI) which we consider as the most suitable expression for chaksine.

It needs only to explain the formation of chaksinic acid (1:2:6-heptane tricarboxylic acid) and 1-methyl pimelio acid by alkali hydrolysis of chaksine. This is indicated in the following steps:
XXXIII \[ \xrightarrow{-H_2 \text{ dehydrogenation}} \] XXXIV

XXXV \[ \xrightarrow{-NH_3 \text{ } \beta\text{-elimination}} \] XXXVI

XXXVII \[ \xrightarrow{\text{laetol formation}} \] XXXVIII

XXXVII \[ \xrightarrow{\text{ketonization}} \] XXXVIII
The first two steps are self-explanatory. The dehydrogenation of the intermediate (XXXIII) to (XXXIV) is supported by the experimental observation that hydrogen is evolved during the fusion. Loss of ammonia from (XXXV) to yield (XXXVI) is readily understandable since it involves a simple $\beta$-elimination. The hydroxy acid (XXXVI) through the lactol formation om
position yields the anhydride (XXXIX), which opens up in alkali to the hydroxy dicarboxylic acid (XL). There is considerable analogy for a reaction of this kind. It is well known\(^{31}\) that maleic acid aldehyde (XLI), on treatment with alkali yields succinic acid (XLII), in a quantitative yield. This reaction can also be assumed to proceed through similar steps.

![Chemical structure](attachment:chemical_structure.png)

The formation of chalconic acid from the hydroxy acid (XL) must involve an oxidation in the presence of alkali at high temperature. The formation of 1-methyl pimelic acid (IV) as the second product during alkaline degradation can be rationalised through the hydration of the intermediate (XXXVI) to the aldol (XLIII) and its subsequent reversal to the hydroxy acid
(XLIV) which undergoes oxidation to yield (IV).

The scheme given above does not imply that the various reactions indicated also follow the order shown here. The actual order may be different.
Dehydrogenation and loss of the guanidine part, opening of the lactone ring, dehydration of the primary alcohol and hydrogenation of the isopropylene to the isopropyl group are steps easy to understand.
Wiesner's structure for chaksine: After our work on chaksine had been concluded and the present manuscript had been typed, we received a communication on the structure of chaksine by Wiesner et al. (1) published in the 20th March, 1958 issue of the Journal of The American Chemical Society (received here on 16th May, 1958). These authors had arrived independently at similar overall conclusions regarding the bicyclic nature of chaksine and the presence of a guanidino function and the incorporation of a large-membered lactone ring in its molecule, though their ultimate choice regarding an expression for chaksine was somewhat different from the one we had considered. The Canadian authors have favoured structure (I) for chaksine - the difference in the two structures is in the size of the ring incorporating the guanididine function.

Their conclusions are based on the two most elegant experiments carried out on chaksine, namely its
controlled hydrolysis by treatment with 2N sodium hydroxide and the isolation of a crystalline ureido hydroxy acid (II) and also the oxidation of the alkaloid with potassium permanganate, followed by decarboxylation and further oxidation with chromic acid to result in the formation of 1-methyl pimelic acid. In our opinion, structure (III) for chaksine, is equally suitable and explains satisfactorily all the important reactions of the alkaloid, like the formation of chaksinic acid by hydrolysis and p-isopropylbenzoic acid by dehydrogenation with copper powder.

The isolation of 1-methyl pimelic acid by oxidation of chaksine with potassium permanganate followed by decarboxylation and further oxidation with chromic acid would imply the formation of the malonic acid (IV), as an
intermediate and this observation apparently lends support to structure (I). However, the formation of such a malonic acid cannot also be ruled out on the basis of the structure (III). The formation of the intermediate (V) during the course of this oxidation appears quite reasonable. Subsequent loss of ammonia through a simple $\beta$-elimination on (V), followed by internal oxidation reduction of the aldehyde hydroxy acid (VI), to the hydroxy dicarboxylic acid (VII), as discussed earlier and further oxidation
to the malonic acid (IV), appears to be a reasonable path for the formation of 1-methyl pimelic acid.

Wiesner's structure also seem to offer the apparent advantage, in explaining the formation of 1-methyl pimelic acid during the alkaline hydrolysis of chaksine, since it involves a simple reversal of the Mannich base as shown in structure (VIII). The formation of chaksinic acid, however, cannot be explained.

\[ \text{COOH} \]
\[ \text{H} \]
\[ \text{H} \]
\[ \text{N} \]
\[ \text{H} \]
\[ \text{NH}_2 \]
\[ \text{HOH}_2C \]
\[ \text{CH}_3 \]

(VIII)

on this mechanism and would probably involve the loss of ammonia through a $\beta$-elimination, followed by oxidation to the unsaturated aldehyde hydroxy carboxylic acid (VI) according to the mechanism already discussed.

Ahmad and Hahn\textsuperscript{30} have demonstrated that there is a marked difference in the rate of evolution of ammonia, during hydrolysis of chaksine by treatment
with boiling baryta solution. Whereas the first mole of ammonia was evolved in 40 hours, the evolution of the second mole was completed only after refluxing for 240 hours. Wiesner's structure (I) should permit the ready evolution of two moles of ammonia by the hydrolysis of the guanidine to the urea and subsequent loss of another mole through a $\beta$-elimination. These results are readily understandable on the basis of the structure (III), for chaksine - where dehydrogenation to the imine followed by hydrolysis to the aldehyde must precede the loss of second molecule of ammonia through a $\beta$-elimination. Wiesner et al.\(^{(1)}\) have also quoted the infrared absorption band at 5.85 $\mu$ (1710 cm$^{-1}$) indicating the presence of a five-membered cyclic urea in case of the ureido hydroxy acid (II) - the corresponding band in the case of 2-methyl imidozolidone (five-membered cyclic urea) being at 1718 cm$^{-1}$. This evidence, however, is inconclusive since the absorption for the corresponding six-membered urea is not known.

It should be possible to distinguish between the two structures by carrying out the usual tests for an $\alpha$ - amino acid on the aminoacidic material also obtained by Wiesner et al.\(^{(1)}\) during the course of the alkaline hydrolysis of chaksine.

EXPERIMENTAL

(All melting points are uncorrected. Infrared spectra were recorded both on Hilger H800 & the Perkin Elmer 21B, double beam recording spectrophotometers. Analyses were carried out by W. Manser, Mikrolaboratorium, Zurich and by Weiler & Strauss, Oxford, England.)

Isolation of chaksine: Ten kilogrammes of the well powdered seeds of Cassia absus freed from husks etc., were extracted with six litres of methanolic hydrochloric acid (0.3 per cent solution) in the cold. Extraction was repeated six to seven times, using fresh solvent each time. The combined extracts were neutralized with ammonia and then acidified with acetic acid and the methanol removed under vacuum. After the last traces of the solvent had been removed, the residual syrup was diluted with three litres of water and extracted thoroughly with ether to remove the fatty material. The aqueous residue was saturated with ammonium sulphate when flocculant chaksine sulphate separated out. This was filtered and then washed with ice cold water. The cake thus obtained was pressed and again washed with acetone followed by ether. After drying in an oven at 100°, there was obtained 221 gms. of the crude chaksine sulphate. Yield 1.95 per cent. This sample was pure enough for conversion to other chaksine salts.
After crystallisation from boiling water, **chaksine sulphate** was obtained as white microcrystalline needles. Melting point 316° (decomp.).

When the aqueous residue as obtained above after extraction with ether, was saturated with ammonium iodide, the corresponding iodide was obtained. **Chaksine iodide** after crystallization from ethanol into white needles, had a melting point 170°.

**Chaksine oxalate** could be precipitated directly from the original methyl alcohol extract by treatment with oxalic acid. After crystallization from boiling water, chaksine oxalate was obtained as a white powder melting point 298° (decomp.).

**Isolation of two crystalline non nitrogenous products from Cassia absus:** The aqueous mother liquor after separation of chaksine sulphate, was allowed to stand at room temperature for several weeks, when a white solid separated at the bottom. This was crystallized from boiling water into sharp needles, melting point 205°.

*Anal. Found:* C, 49.73; H, 7.65.

Similarly the combined ether extracts obtained during the removal of the fatty material was concentrated
till all the ether had been removed. The residual oily material was allowed to stand in the refrigerator for several weeks, when a small amount of solid material had collected at the bottom. The solid mass was separated by filtration and washed with petroleum ether to remove all the sticky oil. It was finally crystallized from boiling ethyl alcohol into microcrystalline powder, melting point 260°.

Anal. Found: C, 71.54; H, 10.36.

Chaksine picrate was prepared by treatment of chaksine iodide with picric acid in boiling ethanol. After recrystallization from ethanol it was obtained as yellow needles melting point 239-240° (decomp.).

Chaksine hydrochloride: To a hot suspension of chaksine sulphate in water (1 gm. in 50 cc.), 2 gms. of powdered barium chloride was added. After boiling for 2 minutes, the precipitated barium sulphate was filtered off and the aqueous filtrate was saturated with sodium chloride. On cooling chaksine hydrochloride separated out. It was filtered and washed with a small quantity of ice cold water. After drying, it was crystallized from a mixture of ethanol and acetone or ethanol and ether, into white needles melting point 178°. A thoroughly
vacuum dried (over phosphorus pentoxide) sample was
analysed.

**Anal.** Found: C, 50.25; H, 7.51; N, 15.97; Cl, 12.98;
0, 12.91. Calcd. for C₁₁H₂₀₂N₃Cl, C, 50.48; H, 7.65;
N, 16.06; Cl, 13.57; 0, 12.24.

**Chaksine nitrate:** Five gms. of chaksine
iodide was dissolved in about 60 cc. of water and the
solution treated with 2 gms. of silver nitrate dissolved
in a small quantity of water. The solution was boiled
and the precipitated silver iodide was removed by fil­
tration. The filtrate on cooling deposited crystals of
chaksine nitrate. These were crystallized from boiling
ethanol into white needles melting point 213°.

**Chaksine bicarbonate:** A cold saturated
solution of potassium bicarbonate was added to a saturated
solution of chaksine iodide in methanol, till no further
precipitation took place. The chaksine bicarbonate thus
obtained was filtered and recrystallized from an aqueous
solution of potassium bicarbonate into a white powder. It
melted at 180° with previous softening.

**Dibenzoyl chaksine:** Five gms. of chaksine
carbonate was suspended in 50 cc. of an aqueous solution
of potassium carbonate and treated with about 10 cc. of
benzoyl chloride. The mixture was thoroughly shaken, till the semisolid mass was converted into a granular solid. This was powdered and thoroughly washed with carbonate solution to free from any unreacted benzoyl chloride. The solid obtained was repeatedly crystallized from dilute alcohol. In this way 1.5 gms. of a micro-crystalline powder melting at 273° was obtained.

Anal. Found: C, 68.69; H, 6.16; N, 9.55. Calcd. for C_{25}H_{27}O_{4}N_{3}, C, 69.28; H, 6.23; N, 9.70.

Reaction of chaksine nitrate with sulphuric acid - isolation of nitrochaksine: Five gms. of chaksine nitrate was slowly stirred into 40 cc. of concentrated sulphuric acid (d, 1.82), keeping the reaction mixture in an ice bath. The reaction mixture was left in a refrigerator for the next 12 hours and then poured into 100 cc. of ice cold water. The precipitated nitrochaksine sulphate was filtered off and washed with a small amount of ice cold water. It was crystallized from dilute alcohol into white needles melting point 170° (decomp.). Guha and Ray reported a melting point 174° (decomp.).

Anal. Found: C, 40.2; H, 6.4; N, 16.3; S, 4.4. Calcd. for (C_{11}H_{19}N_{4}O_{4})_{2}SO_{4} H_{2}O; C, 40.3; H, 6.1; N, 17.1; S, 4.9.
The sulphate could not be obtained in a completely anhydrous condition — even after prolonged drying over phosphorus pentoxide under high vacuum.

When nitrochaksine sulphate was thoroughly triturred with a dilute aqueous solution of sodium carbonate, the corresponding free base was obtained. It was extracted with chloroform and after removal of the solvent the residual solid was crystallized from a mixture of chloroform and ethanol into microcrystalline needles melting point $171^\circ$ (decomp.). Guha and Ray$^8$ reported a melting point $170^\circ$ (decomp.) for this compound.

When nitrochaksine as obtained above was suspended in water and treated with a few drops of concentrated sulphuric acid, the base went into solution. After cooling and trituration, the corresponding sulphate separated out. It was found to be identical with the sulphate obtained directly on dilution.

Similarly when an alcoholic solution of nitrochaksine was treated with excess of oxalic acid, the corresponding oxalate separated out. This was crystallized from boiling water into a white powder melting point $198^\circ$ (decomp.). Guha and Ray$^8$ reported a melting point $205^\circ$ for the oxalate.
The acidic filtrate from which the precipitated
chaksine sulphate had been removed, on neutralisation with
sodium bicarbonate in the cold gave nitrochaksine base,
which after crystallisation from a mixture of chloroform
and ethanol had a melting point 171° (decomp.) and was
undepressed on admixture with a sample of the free base
as obtained above.

Alkaline hydrolysis of nitrochaksine: Five gms. of nitro-
chaksine was suspended in 60 cc. of 10 per cent solution
of sodium carbonate. The mixture was gently warmed on a
steam bath when dissolution slowly took place to yield an
yellow coloured solution. This was allowed to stand at
room temperature for 3-4 hours and then acidified with
hydrochloric acid when a pale yellow solid mass was
precipitated. This compound proved to be acidic in
nature and dissolved in sodium bicarbonate with effor-
vescence. The hydrolysis could also be effected imme-
diately, by treatment with a dilute solution of sodium
hydroxide in the cold. All attempts at crystallization
of this acidic material have failed so far - the substance undergoes extensive decomposition on boiling in different solvents. On treatment with excess of diazomethane, the acidic material was converted into a neutral product, which no longer gave any effervescence with sodium bicarbonate. This neutral product has also not been obtained crystalline so far.

Alkaline degradation of chaksine: Fifteen gms. of chaksine iodide was heated with 50 gms. of potassium hydroxide dissolved in 100 cc. of water, in a thick walled pyrex flask on a metal bath, with arrangements for the collection of the distillates and the gases. The temperature of the bath was slowly raised, permitting the continued distillation of water and increase in the concentration of the alkali. After boiling for some time, the chaksine base went into solution and the liberation of ammonia gas started. When the temperature of the metal bath had reached 300°, there was sudden frothing and in addition to ammonia, hydrogen gas was also evolved. The reaction mixture was maintained at 320 - 340° for about 10 minutes, when the evolution of the gases had subsided considerably and the reaction mixture had settled as a sticky paste. Heating was continued for another 10-15 minutes, until no more gases were evolved. The contents
of the flask after cooling were dissolved in a small amount of water and acidified strongly with hydrochloric acid and extracted with about 500 cc. of ethyl acetate in five lots. The extract was dried over anhydrous magnesium sulphate and the solvent was distilled off—the last traces were removed under high vacuum and heating on a steam bath. The residual oil proved to be a mixture of 1-methyl pimelic acid (IV) and 1:2:6-heptane tricarboxylic acid (XXIX) and was separated as discussed below.

Isolation of 1-methyl pimelic acid (IV): The mixture of acids as obtained above was dissolved in ether and treated with excess diazomethane in the cold. Five and a quarter gms. of the neutral ester thus obtained was subjected to distillation under vacuum and resolved into two fractions (i) 3.2 gms. of an ester boiling at 70-74°/0.5 mm. and (ii) 1.8 gms. of an ester boiling at 110-112°/0.5 mm. The lower boiling fraction was refluxed with 100 cc. of 10 per cent solution of sodium hydroxide for about 10 hours. After cooling, it was acidified with hydrochloric acid and exhaustively extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulphate and the solvent distilled off. The residue distilled at 150°/3 mm., and the distillate on cooling and scratching, deposited a solid mass, which had a melting point 52-55°. It could be
crystallized from a mixture of ethyl acetate and cyclo-
hexane into a white microcrystalline powder melting at
55-57°.

Anal. Found: C, 61.97; H, 7.84. Calcd. for
C₉H₁₄O₄, C, 62.14; H, 7.76.

The di p-toludide obtained through the acid
chloride by treatment with p-toludine in the usual manner,
after crystallization from dilute ethanol had a melting
point 162°.

for C₂₂H₂₈O₂N₂, C, 75.00; H, 7.95; N, 7.95.

The mono acid p-toludide, was obtained by the
partial hydrolysis of the ditoludide. One gm. of the
latter was refluxed with 50 cc. of 5 per cent aqueous
sodium hydroxide solution for 4 hours. Any unhydrolysed
material was removed by filtration and the filtrate was
acidified with hydrochloric acid and extracted with ethyl
acetate. The extract was dried over anhydrous magnesium
sulphate and the solvent removed under vacuum. The resi-
dual mass was crystallized from dilute alcohol into white
needles, melting point 103°.
Anal. Found: C, 68.31; H, 8.24; N, 5.38. Calcd. for C15H21NO3, C, 68.44; H, 8.00; N, 5.32.

Molecular weight (East's) 251.5. Calcd. for, 263.

The dianilide, also prepared in the usual manner had a melting point 161°.

Cyclization of 1-methyl pimelic acid to 1-methyl cyclohexanone: Two gms. of 1-methyl pimelic acid was thoroughly mixed with 5 gms. of calcium oxide and the mixture subjected to distillation, when about 0.4 gm. of a liquid boiling point 175-177° was obtained. It proved to be 1-methyl cyclohexanone.

The dinitrophenylhydrazone, prepared in the usual manner, after crystallization from ethanol had a melting point 134-136°, alone or on admixture with an authentic sample.

The semicarbazone, had a melting point 192-194°, which was also undepressed on mixing with an authentic sample.

Synthesis of 1-methyl pimelic acid: Thirty two gms. of 2-carboethoxy cyclohexanone (II), was added to a well stirred boiling suspension of 4.65 gms. of sodium hydride
in 250 cc. of dry toluene. Reﬂuxing with stirring was continued until no more hydrogen was evolved. At this stage 40 gms. of methyl iodide dissolved in 50 cc. of toluene was added slowly over a period of 30 minutes. Reﬂuxing was continued for another three hours. The reaction mixture was then cooled, and treated with excess of water. The organic layer was separated, washed with water and dried over anhydrous magnesium sulphate. Toluene was distilled off and the residual oil fractionated, when 27 gms. of 2-methyl-2-carboethoxy cyclohexanone (III) boiling point 68-70°/0.4 mm. was obtained (reported\textsuperscript{34} boiling point 96-100°/4 mm.).

In order to effect the ring opening, 10 gms. of the ester obtained as above was added to a solution of 9.6 gms. of sodium hydroxide dissolved in 40 cc. of absolute methanol. The mixture was reﬂuxed at 120° for 4-5 hours. Fifty cc. of water was then added and excess of methanol was removed under vacuum. The aqueous solution was strongly acidiﬁed with hydrochloric acid and 1-methyl pimelic acid was extracted with ethyl acetate. After distillation of the solvent, the residue was crystallized from a mixture of ethyl acetate-cyclohexane, into white powder melting point 54-56°.
The dianilide and ditoludide prepared in the usual manner had melting point 161° and 162° respectively and there was no depression in the mixture melting points on admixture with the samples prepared from the natural acid.

The isolation of chaksinic acid: Five gms. of the higher boiling ester fraction as obtained above, was refluxed with 100 cc. of 10 per cent solution of sodium hydroxide for 10-12 hours. The cooled reaction mixture was then acidified strongly with hydrochloric acid and extracted thoroughly with ethyl acetate. After drying and removal of the solvent, 2.8 gms. of a thick viscous liquid was obtained. This was treated with a small quantity of ice cold ether and seeded with a few crystals of chaksinic acid. The solid acid separated out. This was filtered and recrystallized twice from a mixture of ethyl acetate - cyclohexane into white needles melting at 145-147°.

Anal. Found: C, 51.72; H, 6.85; neutral equivalent 77.6. Calcd. for C_{10}H_{16}O_{6}, C, 51.70; H, 6.90; neutral equivalent 77.7.

The tri-p-toludide of this tricarboxylic acid, prepared through the acid chloride, by interaction with phosphorus pentachloride in the usual manner and subsequent
treatment with p-toludine was crystallized from dilute alcohol into a white powder, melting point 245°.

Anal. Found: C, 74.61; H, 7.48; N, 8.07; Molecular weight (Rast's) 393. Calcd. for $C_{31}H_{37}N_3O_3$, C, 74.55; H, 7.41; N, 8.42. Molecular weight 399.

Chaksinic acid anhydride: Three gms. of chaksinic acid was heated at 160-170° for an hour and was subsequently distilled under vacuum to yield about 2 gms. of the acid anhydride, boiling at 210-212°/0.1 mm. It was a colourless thick viscous liquid.

Anal. Found: C, 54.53; H, 6.72. Calcd. for $C_{10}H_{14}O_5$, C, 56.07; H, 6.54.

Synthesis of 113:7-heptane tricarboxylic acid (V).

2-carboethoxy-2-ethyl cyclohexanone propionate (VII): In a three-necked flask fitted with an efficient stirrer and condenser, protected from moisture, were placed 400 cc. of dry toluene and 7 gms. of powdered sodium metal. Forty four gms. of 2-carboethoxy cyclohexanone(II), was run in with stirring. Refluxing was continued until the evolution of hydrogen had ceased. At this stage 38 gms. of ethyl-$\beta$-bromo propionate was slowly run in. The mixture was
refluxed for an additional 3-4 hours. After cooling, water was added. The organic layer was separated and washed with water. After drying over anhydrous magnesium sulphate and removal of toluene, the residue was fractionated to yield 28 gms. of the desired product boiling at 128-130°/0.2 mm.

The same ester was obtained when ethyl acrylate was condensed with 2-carboethoxy cyclohexanone in the presence of 0.2 equivalent of sodium ethoxide in absolute ethanol.

Ring opening of 2-carboethoxy-2-ethyl cyclohexanone propionate (VII): Five gms. of the diester obtained as above, was slowly added to a boiling solution made by dissolving 4 gms. of sodium hydroxide in 25 cc. of anhydrous methanol. The mixture was refluxed for about four hours. Hundred cc. of water was then added and the methanol was distilled off. The solution was further concentrated to about 30 cc. and then acidified with hydrochloric acid. After exhaustive extraction with ethyl acetate, the extract was dried over magnesium sulphate, and ethyl acetate distilled off. The residual oil was distilled under high vacuum to yield 2 gms. of 1:3:7-heptane tricarboxylic acid. The distillate solidified
after trituration and was crystallized from a small amount of ethyl acetate containing cyclohexane into a white powder melting at 78\(^\circ\).

**Anal.** Found: C, 51.45; H, 6.82. Calcd. for C\(_{10}H_{16}O_6\), C, 51.70; H, 6.90.

The tritoludide, obtained in the usual manner was crystallized from dilute alcohol and melted at 215-216\(^\circ\).

**Anal.** Found: C, 74.41; H, 7.33; N, 8.45. Calcd. for \(C_{31}H_{37}N_3O_3\), C, 74.55; H, 7.41; N, 8.42.

**Syntheses of 1:2:7-heptane tricarboxylic acid (XI)**

1) **Starting from pimelic acid:**

**Ethyl hydrogen pimelate:** This was carried out according to the procedure recommended for ethyl hydrogen sebacate\(^{35}\). Eighty three gms. of diethyl pimelate, 104 gms. of pimelic acid, 33 cc. of di-n-butyl ether and 17 cc. of concentrated hydrochloric acid were refluxed at 160-170\(^\circ\) till the mixture became homogeneous. The temperature of the bath was lowered to 120-130\(^\circ\) and 39 cc. of 95 per cent ethyl
alcohol was added and refluxing was continued for another 2 hours. At this stage another 13 cc. of ethyl alcohol was added and the mixture was further refluxed for 2 hours. The entire mixture was fractionated to yield 80 gms. of ethyl hydrogen pimelate, boiling point 140-142°/0.4 mm.

Pimelic aldehyde mono ethyl ester (XII) (Rosenmund reduction): In a three necked flask equipped with a mercury sealed stirrer, a reflux condenser carrying an outlet tube and arrangements for leading hydrogen gas, was placed 85.5 gms. of the distilled acid chloride (obtained from ethyl hydrogen pimelate and thionyl chloride) dissolved in 250 cc. of dry xylene. Eight gms. of 10 per cent palladium-barium sulphate catalyst was added along with 0.5 cc. of the quinoline sulphur poison. The flask was heated to 140-145 and hydrogen gas was let in. The hydrogen chloride liberated was passed into 190 cc. of 2N sodium hydroxide solution containing a few drops of phenolphthalein. Reduction was continued till the sodium hydroxide solution was neutralized. At this stage evolution of hydrogen chloride had slackened considerably. Hydrogenation was discontinued and after cooling the catalyst was filtered off. Fractionation of the filtrate gave the desired aldehyde.
Boiling point 93-94°/0.2 mm., yield 53 gms.

The yellow 2:4-dinitrophenylhydrazone, after crystallization from alcohol, had a melting point 172-173° (decomp.).


1-heptene-1:7-dicarboxylic acid (XIII): A mixture of 53 gms. of the aldehyde ester obtained as above, 70 cc. of pyridine, 1.6 cc. of piperidine and 62.5 gms. of malonic acid was heated on a water bath, until there was no effervescence. It was allowed to stand at room temperature for the next 12 hours and then diluted with 200 cc. of water. It was decomposed with hydrochloric acid and after extraction with ether, drying over anhydrous magnesium sulphate etc., the solvent was removed and the residue distilled under vacuum when the half acid ester boiling point 158-160°/0.2 mm. was obtained. Yield 37 gms.

Further hydrolysis was carried out with excess of 15 per cent sodium hydroxide solution. The reaction mixture was worked out in the usual manner, when 5.5 gms. of 1-heptene-1:7-dicarboxylic acid was obtained. After crystallization from water into white needles, it had a melting point 101-102°.
Anal. Found: C, 57.07; H, 7.32. Calcd. for C_{9}H_{14}O_{4}, C, 58.06; H, 7.52.

1-heptene-ethyl-1:7-dicarboxylate (XIV): The dicarboxylic acid from above, was converted into its acid chloride by treatment with oxalyl chloride in anhydrous benzene. The diacid chloride was further reacted with ethyl alcohol to form the diester, boiling point 122-124^\circ/0.1 \text{ mm}.

2-cyano-ethyl 1:7-heptane dicarboxylate (XV): To a refluxing solution of 3 gms. of sodium cyanide in 12 cc. of water and 25 cc. of 95 per cent ethyl alcohol was added 12 gms. of ethyl 1-heptene-1:7-dicarboxylate. The mixture was refluxed for about 3 hours and acidified with hydrochloric acid. Alcohol was then distilled off and the mixture was extracted with ether. The extract was washed with water and dried over anhydrous magnesium sulphate. After removal of the ether, the residual oil was fractionally distilled to yield 8.2 gms. of the cyano ester, boiling point 180-184^\circ/0.2 \text{ mm}.

Hydrolysis of 2-cyano-ethyl 1:7-heptane dicarboxylate to 1:2:7-heptane tricarboxylic acid (XI): cyano ester was refluxed with 40 cc. of concentrated
hydrochloric acid for 5 hours. After drying on a water bath under suction, the residue was extracted with ethyl acetate to yield 2.4 gms. of a solid. This proved to be a mixture of 1:2:7-heptane tricarboxylic acid and some unreacted 1-heptene-ethyl 1:7-dicarboxylate. The separation was quite wasteful. Repeated crystallization from a mixture of ether-benzene gave a small amount of 1:2:7-heptane tricarboxylic acid, melting point 77°C.

(ii) Starting from cycloheptanone:

2-carboethoxy cycloheptanone (IX): Twenty five gms. of cycloheptanone (VIII) was added to a well-stirred suspension of 10.7 gms. of sodium hydride in 200 cc. of dry thiophene free benzene, containing 52.71 gms. of diethyl carbonate. The mixture was gently refluxed during the course of addition and further refluxed for 15-16 hours. During this time considerable amount of the solid sodium salt had separated out. The reaction mixture was cooled and excess of sodium hydride was destroyed by addition of ethyl alcohol. It was then decomposed with slight excess of ice cold dilute hydrochloric acid. The organic layer was separated, washed successively with bicarbonate solution and water. After drying over anhydrous magnesium sulphate
and removal of benzene, the residue was subjected to vacuum distillation to yield 9.3 gms. of the desired product, boiling point 71⁰/0.2 mm. The above mentioned procedure should be preferred to that described by Prelog and Henden³⁷.

2-carboethoxy-2-ethyl cycloheptanone acetate (X): To a well-stirred suspension of 1.22 gms. of sodium hydride in 150 cc. of dry benzene, was added 9.3 gms. of 2-carboethoxy cycloheptanone (IX). The mixture was refluxed until the evolution of hydrogen had ceased. After this 10.1 gms. of ethyl bromo acetate was added during 30-40 minutes to the refluxing mixture. After 3 hours, the reaction mixture was cooled and cautiously decomposed with ice cold water. The benzene layer was separated, washed with water and dried over anhydrous magnesium sulphate. Distillation under vacuum yielded 5.5 gms. of the diester, boiling point 115-117⁰/0.1 mm.

**Anal. Found:** C, 62.05; H, 8.28. Calcd. for C₁₄H₂₂O₅, C, 62.22; H, 8.15.

The ring opening and hydrolysis of the above diester was carried in a manner similar to that described under 1:3:7-heptane tricarboxylic acid. 1:2:7-heptane
tricarboxylic acid was crystallized from ethyl acetate into white microcrystalline powder, melting point 76-77°. Mixture melting point with a sample obtained from pimelic acid etc. as described above was not depressed.


The tritoludide was crystallized from dilute alcohol into white needles, melting point 194-195°.

Anal. Found: C, 74.17; H, 7.85; N, 8.34. Calcd. for \( \text{C}_{31}\text{H}_{37}\text{N}_3\text{O}_3 \), C, 74.55; H, 7.41; N, 8.42.

**Synthesis of 1:4:7-heptane tricarboxylic acid (XIX)**

2-carboethoxy-2-ethyl cyclopentanone crotonate (XVII): The condensation of 2-carboethoxy cyclopentanone\(^{38}\) with ethyl-Y-bromocrotonate, was carried out in the usual manner with sodium hydride in boiling toluene. The condensation product boiled at 171-173°/4 mm.

2-carboethoxy-2-ethyl cyclopentanone butrate (XVIII): Eighteen gms. of 2-carboethoxy-2-ethyl cyclopentanone
crotonate (XVII), was dissolved in 200 cc. of ethyl alcohol and hydrogenated in the presence of 2 gms. of 10 per cent palladium strontium carbonate catalyst at 50 pounds pressure. After absorption of one mole of hydrogen, the reaction was stopped. The catalyst was filtered and the filtrate was fractionated to yield 16.5 gms. of the desired ester, boiling point 164-165°/4 mm.

Bachmann who also prepared this ester reported a boiling point 140-145°/0.4 mm.

The ring opening and hydrolysis was carried out by boiling methanolic sodium hydroxide in the usual manner. 1:4:7-heptane tricarboxylic acid was crystallized from ethyl acetate into white needles, melting point 120-121°.


The tritoludide prepared through the acid chloride was crystallized from dilute alcohol, melting point 225°.

Anal. Found: C, 74.32; H, 7.2; N, 8.25. Calcd. for \( \text{C}_{31}\text{H}_{37}\text{N}_3\text{O}_3 \): C, 74.55; H, 7.41; N, 8.42.
Synthesis of 2-methyl-2'-carboxy suberic acid (XXVI).

$\delta$-acetyl-$\alpha$-valeric acid: This was prepared by the oxidation of o-methyl cyclohexanone (XXI) by chromic acid according to the directions of Schaeffer$^{40}$. The methyl ester (XXIII) of this acid was prepared by using methanol and sulphuric acid in the usual manner.

Methyl-1-cyano-2-methyl-$\alpha,\beta$-hexene-1:6-carboxylate: A mixture of 100 gms. of the above ester, 63 gms. of methyl cyanoacetate, 3 gms. of ammonium acetate, 7 cc. of glacial acetic acid and 20 gms. of acetamide in 400 cc. of benzene was heated under reflux using a water separator. After 3 hours when 10.5 cc. of water had separated, heating was discontinued. The reaction mixture was washed with water and the organic layer was dried over anhydrous magnesium sulphate. After removal of the benzene, the residue was distilled to yield 72 gms. of the desired unsaturated cyano compound (XXIV), boiling point 145-147$^0$/0.1 mm. It was pure enough to be used in the next step directly.

Dimethyl-1:2-dicyano-2-methyl suberate: Sixty gms. of the above ester (XXIV) was dissolved in 300 cc. of
95 per cent ethyl alcohol and treated with 27 gms. of sodium cyanide dissolved in 200 cc. of water — the reaction mixture was cooled to 10° during the addition. It was then acidified with hydrochloric acid and allowed to stand for 2 hours. The reaction mixture was further diluted with 1250 cc. of dilute hydrochloric acid and the lower layer separated. The aqueous solution was twice extracted with 250 cc. of ether. The total extract was washed with water and dried over anhydrous magnesium sulphate. After removal of ether the residue was fractionated to yield 51 gms. of the desired dinitrile (XXV), boiling point 180-182°/0.1 mm.

Hydrolysis of dimethyl-1:2-dicyano-2-methyl suberate to 2-methyl-2'-carboxyl suberic acid: Fifteen gms. of the dinitrile was refluxed with 60 cc. of concentrated hydrochloric acid dissolved in 10 cc. of water for 16 hours. At the end of the reaction, hydrochloric acid was completely removed under vacuum and the residue was extracted with ethyl acetate. After removal of the solvent, the tricarboxylic acid was recrystallized from boiling concentrated hydrochloric acid into white needles, melting at 117-118°. Yield 8.5 gms.

The synthesis of chaksinic acid (1:2:6-heptane tricarboxylic acid XXIX)

2-methyl-6-carboethoxy cyclohexanone (XXVII): The condensation of o-methyl cyclohexanone and ethyl oxalate was carried out in a manner identical to that used for the condensation of cyclohexanone and ethyl oxalate. The subsequent pyrolysis to remove carbon monoxide was also carried out similarly. 2-methyl-6-carboethoxy cyclohexanone (XXVII) boiled at 115-117°/12-13 mm.

2-methyl-6-carboethoxy cyclohexanone-6-ethyl acetate (XXVIII): Four gms. of potassium metal was dissolved by refluxing in 150 cc. of dry tertiary butyl alcohol. After complete dissolution had taken place, 18.5 gms. of 2-methyl-6-carboethoxy cyclohexanone was added and the mixture was further refluxed for another 15 minutes. After this 16.7 gms. of ethyl bromoacetate was added over a period of 30 minutes and the mixture was refluxed for another 3 hours. After allowing it to stand overnight, the excess of tertiary butyl alcohol was distilled off and the residue was diluted with water and then acidified with hydrochloric acid. It was extracted with ether and then dried over anhydrous magnesium sulphate. After removal of the ether, the residue was distilled under vacuum to yield
18 gms. of the desired diester, boiling point 132-133°/1.5 gms.

Ring opening and hydrolysis of 2-methyl-6-carboethoxy cyclohexanone-6-ethyl acetate to 1:2:6-heptane tricarboxylic acid: Ten gms. of the diester was added to a refluxing sodium methoxide solution, prepared by dissolving 2 gms. of sodium metal in 100 cc. of absolute methanol. The sodium salt separated out at once. Refluxing was continued for another 2 hours. Excess of alcohol was distilled off and the residue was diluted with 50 cc. of water. It was further refluxed for about 2 hours and then strongly acidified with hydrochloric acid. The triacid was extracted with ethyl acetate. After drying over anhydrous magnesium sulphate and removal of ethyl acetate, there was obtained a thick viscous oil. This was seeded with solid chaksinic acid, when the entire mass solidified. It was triturated with ether and the solid acid was filtered. Yield 4.8 gms. After two crystallizations from a mixture of ethyl acetate and cyclohexane, 1:2:6-heptane tricarboxylic acid was obtained as a microcrystalline powder melting point 145-147°, alone or on admixture with the natural sample.

The tritoludide, prepared in the usual manner, had a melting point 245° and was not depressed on mixing with a sample prepared from the natural acid.