CHAPTER 3

CHEMICAL INVESTIGATION OF Datura Quercifolia HBK

INTRODUCTION 63

Datura Quercifolia HBK 64

REVIEW OF LITERATURE 65

WITHANOLIDES 67

STRUCTURE ELUCIDATION OF COMPOUNDS OF Datura Quercifolia HBK 69

Dq-2
Solubility, p.70; Tests for elements and functional groups (chemical methods), p.70; Element analysis, p.71; Specific rotation, p.72; Spectral data, p.72; Dq-2 hydrogenated, p.77; Conclusion, p.78.

Dq-3
Solubility, p.79; Tests for elements and functional groups (chemical methods), p.79; Spectral data, p.79; Conclusion, p.82.

Dq-4
Solubility, p.83; Tests for elements and functional groups (chemical methods), p.83; Element analysis, p.83; Acetylation, p.83.

Dq-5
Solubility, p.84; Element analysis, p.84.

EXPERIMENTAL SECTION 85

REFERENCES 89
Datura is a small genus of about twelve species, distributed throughout the tropical and warm temperate regions of the world. Some of the species are cultivated for ornamental purposes.

The plants are coarse, rank-scented, shrub-like herbs or small trees with entire or coarsely sinuate-dentate leaves. Flowers large, white or purple, solitary, calyx long, tubular, herbaceous, 5-toothed, breaking transversely above the base in fruit. Corolla tubular, funnel shaped. Stamens attached near the corolla-base, included. Capsule ovoid or glabose, usually spinous, 4-celled, or 4-valved or irregularly breaking up near the apex. Seeds compressed, rugose or dotted.

Datura has been known for ages because of its narcotic and hypnotic effects. These effects have been utilized in religious ceremonies, in oracular divination or in forecasting of future events and in this respect all the species seem to have the same properties. In western South America, the herb was used by natives to induce partial intoxication, to control unruly children and mixed with tobacco given to women and slaves to deaden their senses before burial alive with their dead husbands or masters. Aztec Indians used the drug for the treatment of all types of diseases including paralysis and priests used the plant in the form of drinks to enable them to communicate with spirits. Some tribes used the plant in ceremonies in initiating boys into manhood.

About 10 species of Datura occur in India and out of these Datura stramonium, D. metel, D. innoxia and D. fastuosa are very
common. The Indian people have long been familiar with the narcotic and intoxicating properties of *Datura*, and in many parts the plant has been regarded as sacred. Thugs have used the drug obtained from *Datura* to stupefy and daze their victims. The drug has also been used for criminal poisoning and as a treatment for hydrophobia.

*D. stramonium* is used in medicine as narcotic, anti-spasmodic, anodyne, treatment of parkinsonism and to relieve the spasm of the bronchioles in asthma. It has also been used for the treatment of haemorrhoids, boils, sores, fish-bites and the juice of flowers is used in ear-ache. Fruit juice is applied to the scalp for curing dandruff and falling hair. *D. innoxia* leaves and flowering tops have been seen to have anti-spasmodic properties and green leaves of *D. metel* are reported to be used for dyeing cloth in East Africa.

**DATURA QUERCIFOLIA HBK**

It is an annual, erect terrestrial herb with a limited range of distribution. It was first found in Mexico and described by Humboldt, Bonpland and Kunth in 1818. In India the plant has been introduced for the first time in Kashmir and has thrived best under the climatic conditions of the valley. It has got an erect 0.5 to 1.5 meter high, slightly downy or pubescent, branched stem. Each fork of the dichotomous stem has a flower bud. Leaves deeply pinnately lobed and slightly downy or pubescent. Flowers large, corolla five-toothed, pale lavender, 4 to 7 cm long and about 2 cm wide. Calyx half as long as the corolla. Anthers purple. Capsule erect, ovoid, 6 to 7 cm long, 4 to 6 cm wide, including spines. Spines
very unequal in size, larger at the top of the capsule and less stout. On ripening capsule breaks into four halves. Seeds black. This species has not been used for hypnotic and narcotic purposes as extensively as other species and has mainly been worked for alkaloids\textsuperscript{5,6}.

The herbarium has been deposited with Regional Research Laboratory, Srinagar, under Field No. RRL-16272.

**REVIEW OF LITERATURE**

**Datura** species have mainly been worked for alkaloids. Chemical examination has shown that it contains alkaloids of tropane class mainly. The most commonly occurring alkaloids are: atropine, hyoscine, L-hyoscyamine, apohyoscyamine, DL-scopolamine, meteloidine, nor-meteloidine, tigloyl-putrescine, scopine, nor-tigloidine, tropine, pseudo valeroidine, fastudine, fastunine, fastusidine, fastusine, fastusinine, etc.\textsuperscript{7} Other alkaloids and compounds isolated from the species have been summed up in the following table:

<table>
<thead>
<tr>
<th>Datura species</th>
<th>compounds isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Datura stramonium</em></td>
<td>Hyoscine-N-oxide\textsuperscript{8}; flavonol glycosides,</td>
</tr>
<tr>
<td></td>
<td>caffeic acid, p-cumaric acid and ferulic acid\textsuperscript{9},</td>
</tr>
<tr>
<td></td>
<td>ascorbic acid; 6-hydroxyhyoscyamine\textsuperscript{10};</td>
</tr>
<tr>
<td></td>
<td>L-ketoglutaric acid and pyruvic acid\textsuperscript{11}.</td>
</tr>
<tr>
<td><em>D. innoxia</em></td>
<td>6β-propanoyloxy-3α-tigloyloxytropane\textsuperscript{12};</td>
</tr>
<tr>
<td></td>
<td>daturadiol and daturaolone\textsuperscript{13}.</td>
</tr>
<tr>
<td>Datura species</td>
<td>Compounds isolated</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>D. metel</strong></td>
<td>7-hydroxy-3,6-ditigloyloxytropane(^1^); daturanolone and fastusic acid(^1^)</td>
</tr>
<tr>
<td><strong>D. candida</strong></td>
<td>Esters of tiglic acid with 3α-hydroxytropane, 3α,6β-dihydroxytropane and 3α,6β,7β-trihydroxytropane(^1^); 3α,6β-ditigloyloxytropan-7β-ol, 3α-tigloyloxytropane and tropine(^1^)</td>
</tr>
<tr>
<td><strong>D. sanguinea</strong></td>
<td>Esters of tiglic acid with 3α-hydroxytropane, 3α,6β-dihydroxytropane, 3α,6β,7β-trihydroxytropane and 3α-tigloyl-6β-acetoxytropane(^1^); 6β-isovaleryloxy-3α-tigloyloxytropan-7β-ol, 3α-tigloyloxytropane and (−)-3α-(3α-hydroxy-β-phenylpropionyloxy)-tropane(littorine)(^1)</td>
</tr>
<tr>
<td><strong>D. arborea &amp; D. meteloides</strong></td>
<td>7-hydroxy-3,6-ditigloyloxytropane(^1).</td>
</tr>
<tr>
<td><strong>D. tatula</strong></td>
<td>Coumarins(^2).</td>
</tr>
<tr>
<td><strong>D. quercifolia</strong></td>
<td>Daturalactone(^2)</td>
</tr>
<tr>
<td><strong>Datura species</strong></td>
<td>1-O-feruloylglucose(^2) and nicotine(^3).</td>
</tr>
</tbody>
</table>
**WITHANOLIDES**

*Datura quercifolia* has recently\(^{21}\) been added to the group of withanolide bearing plants of *Solanaceae*. Withanolides are a series of related steroidal lactones. They have been named to reflect the botanical origin of the first member of the series, withaferin A\(^{24}\), from *Withania somnifera*. They possess an ergostane skeleton in which a six-membered-ring-22-hydroxy-26-oic acid lactone is present. Withanolides differ from each other in the position and configuration of various substituents, especially -OH, double bond and epoxide. Most of the withanolides contain a 1 enone system and R configuration at C-20 and C-22 positions. One or more -OH groups are usually present at 4β, 5α, 7α, 12β, 14α, 17β, 27-CH\(_2\)OH and 20β positions; withanolides, containing a 20β-OH, contain S configuration at C-20 position. The most common positions for double bonds are: 2,3; 3,4; 5,6; 8,14; 14,15; 27,28 etc., and the epoxide is usually present at one of the positions 5β-6β, 6α-7α and 14-15.

More than 35 compounds of withanolide class have been isolated so far\(^ {25-34}\) from different plants of *Solanaceae* and structures (a), (b), (c) and (d) present a representative range.

(a) ![Withanolide Structure](image)

(b) ![Withanolide Structure](image)
Vithanolides are known to possess anti-tumor activity. Withaferin A shows significant inhibitory activity against the SA tumor in mice and the Walker intramuscular carcinosaoma 256 (WM) in rats. Withaferin A and withacnistin also show cytotoxicity against KB cell cultures.
STRUCTURE ELUCIDATION OF

COMPOUNDS OF DATURA QUERCIFOLIA HBK

Fresh leaves of *Datura quercifolia* HBK were extracted with cold benzene. The extract on concentration deposited a crystalline residue (Dq-1). After separation of the crystalline residue the mother liquor was concentrated and subjected to chromatography on a column of silica gel, using benzene and a linear gradient of benzene against EtOAc as eluents. This provided cuts I (least polar) and II (polar) according to TLC behaviour.

Cut II was again subjected to column chromatography over silica gel using benzene and benzene - EtOAc as eluents. Various sections of the column were suitably pooled and the following compounds obtained in order of decreasing Rf. values:

Dq-3
Dq-2
Dq-1 - This compound was found to be daturalactone.²¹

Cut I was also subjected to column chromatography over silica gel using light petrol, light petrol - benzene and benzene as eluents. Various fractions were pooled according to TLC behaviour. The following compounds were obtained:

Dq-5
Dq-4
**Dq-2**

**Solubility:**

Dq-2 is soluble in benzene, CHCl₃, ether and alcohol and insoluble in light petrol, water, 5% aq. NaHCO₃, 5% aq. NaOH, 5% aq. HCl and 85% aq. phosphoric acid solutions. It dissolves in cold conc. H₂SO₄ giving a deep blue solution. The compound thus falls in group "N₂", neutral, class of compounds according to Shriner, Fison and Curtin.

**Tests for elements and functional groups (chemical methods):**

1. **Detection of elements:** It showed the absence of N, S and halogens in the Lassaigne's test.

2. **Free -COOH group:** The compound gave no effervescence with aq. NaHCO₃ solution. This shows the absence of a free -COOH group.

3. **Phenolic -OH group:** Dq-2 did not give any colour with aq. or alcoholic 1% FeCl₃ solution. This shows the absence of phenolic -OH group in the molecule.

4. (a) **Methylenedioxy group:** The compound did not show any colour in Labat and Gaebel tests - methylenedioxy group absent.

   (b) **Methoxyl group:** The compound did not liberate HCHO on heating with benzoyl peroxide.

5. **Epoxide group:**

   (a) Added 2ml of conc. HNO₃ and 2ml 10% AgNO₃ solution to 25ml of 2% pot. periodate solution. Filtered the solution. Took 10mg of Dq-2 in 1ml of dioxan and added one drop of the above
reagent to one drop of the solution of Dq-2 in dioxan. A light yellow turbidity appeared.

(b) 10mg of the compound in acetone (1.5ml) were treated with 0.1N solution of Na₂S₂O₃ (0.5ml) and one drop of phenolphthalein. A permanent red colour on warming indicated the presence of an epoxide group. The blank test did not show any red colour.

7. Lieberman - Burchard test :- Dq-2 gave a positive test for steroids.

8. Acetylation :- Dq-2 was treated with a 1:1 mixture of acetic anhydride and pyridine at 28° for 30 hr. The mixture was poured in ice-cold water with stirring. The water extract was extracted with CHCl₃. CHCl₃ extract was washed with water, dried over Na₂SO₄ and evaporated to dryness. A white solid which was found to be identical with Dq-2 in all respects appeared. This shows that Dq-2 does not contain a primary or secondary -OH group.

The information gathered so far indicates that Dq-2 contains the following functionalities:

1. It is a steroid.
2. It contains an epoxide group or groups.
3. It does not contain a primary or a secondary alcoholic group.

Element analysis :-

Found: C, 67.1; H, 7.51.

Calculated for C₂₈H₃₆O₇: C, 67.35; H, 7.43%.

The molecular formula suggests that the total number of rings and sites of unsaturation present in the molecule is eleven.
Specific rotation: -

\[ [\alpha]_D^{18} = +63 \text{ (C, 1.0; CHCl}_3 \text{)} \]

Spectral data: -

UV spectrum: - UV spectrum shows a strong absorption at 225 nm. This shows the presence of an \( \alpha, \beta \)-unsaturated ketone or lactone chromophore or both.

IR spectrum (Fig.1): - IR shows principal bands at 3526, 3510, 2982, 2943, 1730, 1700, 1683, 1460, 1388, 1303, 1140, 1088, 905 cm\(^{-1}\), etc.

The peaks at 3526 and 3510 cm\(^{-1}\) show the presence of two \(-\text{OH}\) groups in the molecule. Since the compound remains unchanged upon attempted acetylation under mild conditions (\(\text{Ac}_2\text{O - pyridine}\)) this shows that both the \(-\text{OH}\) groups are tertiary in nature. The peak at 1683 cm\(^{-1}\) is due to the unsaturated ketone group and the one at 1700 cm\(^{-1}\) can be attributed to a saturated six-membered ring ketone. The band at 1730 cm\(^{-1}\) shows the presence of a six-membered ring \(\alpha\)-lactone with unsaturation \(\alpha\) to \(\gamma\text{C=O}\) group.

Out of the seven oxygen atoms in the Dq-2 molecule IR accounts for the presence of six atoms, and since the compound gives a positive test for epoxide group the 7th oxygen must be present as an epoxide in the molecule.

NMR spectra (Fig.2): - The NMR spectrum of Dq-2 is recorded in table 1 along with proton assignments. Most of the signals of Dq-2 are similar to the corresponding signals of withanolides. The low field double quartet at \(\delta\ 6.60\ (10, 4.5, 3\text{Hz})\) and a doublet showing a weak allylic coupling at \(\delta\ 5.85\ (10\text{Hz})\) indicate an ABXY type of system in the molecule. This type of system is only
possible when the \( \alpha, \beta \)-unsaturated ketone group is present in ring A. The ketone group of the enone system can either be kept at 1 or 4 position. The low field signal of C-19 protons at \( \delta \) 1.26 suggests that the \( \rangle C=0 \) is at position 1; had it been at position 4 the C-19 protons should have been at a position up field by 0.408 ppm \( (0.375 + 0.033 \text{ppm})^{40} \) from the present position. The doublet at \( \delta \) 5.85 (showing a weak allylic coupling) can, therefore, be attributed to C-2 proton and the dq at \( \delta \) 6.60 to C-3 proton.

Out of the three different carbonyl functions shown by IR spectrum, the position of \( \alpha, \beta \)-unsaturated ketone has been fixed by NMR. The only available positions for six-membered ring ketone are in rings B and C at 6,7,11 or 12 positions. The effect of placing \( \rangle C=0 \) at each of these positions on C-18 and C-19 protons can be considered as: If \( \rangle C=0 \) is kept at C-6 position it will move the C-19 proton signal up field by 0.05ppm and C-18 proton signal down field by 0.017ppm from the observed values of \( \delta \) 0.792 for C-19 and \( \delta \) 0.692 for C-18 protons of 5\( \alpha \), 14\( \alpha \)-androstane.\(^{40}\) The C-7 \( \rangle C=0 \) will move the C-19 and C-18 proton signals down field by 0.275 and 0.008ppm\(^{41} \), respectively. From the observed chemical shifts of C-19 and C-18 protons it is clear that none of these positions account for the low field positions of these protons. Now the only position for \( \rangle C=0 \) can either be at C-11 or C-12. A C-11 keto function will deshield the C-19 protons and shield the C-18 protons, whereas C-12 \( \rangle C=0 \) will deshield the C-18 protons more \( (0.375 \text{ppm})^{42} \) than C-19 \( (0.1 \text{ppm}) \) protons. The observed position of \( \delta \) 1.1 for C-18
protons reflects the contribution of a C-12 ketone.

The C-12 assignment is further supported by comparing the chemical shifts of C-18 and C-21 methyl protons of Dq-2 with withanolides\textsuperscript{27} devoid of ring C keto functions. The significant difference between the positions of these protons which appear very much down field (C-18) in Dq-2 can only be explained when $\mathrm{\text{C}=0}$ is kept at 12 position. This is the only position which can simultaneously deshield C-18 and shield C-21 protons.

The signal patterns of the two doublets at 6 3.08 (4Hz) and 3.4 (4:1Hz, broadened and unresolved) suggest two epoxide protons incorporated in a system of the type:

![Chemical Structure]

This system is possible either in ring B (a or b) or ring D (c or d). The J values of the doublets, 4Hz, exclude the possibility of a 5-membered ring epoxide (vicinal coupling constants between epoxy protons in 1,2-epoxycyclopentane system is equal to about 3Hz).\textsuperscript{43}

Out of the two possibilities a and b in ring B, the former is more appropriate and in agreement with the observed chemical shift of C-19 protons by 0.183ppm.\textsuperscript{41}

The stereochemistry of the epoxide can be put as $\alpha$ or $\beta$. From the study of molecular models and by analogy with other withanolides\textsuperscript{29} containing 6,7 $\alpha$-epoxides, it is clear that the stereochemistry of the epoxide is $\alpha$. 

\[ \text{Chemical Structure} \]

\[ \text{Molecular Model} \]
The above assignments by NMR are also in perfect agreement with the NMR values of withanicandrin, the first naturally occurring 12-oxowithanolide.

The doublet at $\delta$ 0.9 (7Hz) is due to C-21 protons coupled to C-20 proton. The C-22 proton couples with C-23 protons of the lactone ring on one side and the C-20 proton on the other side, and, therefore, appears as a multiplet at $\delta$ 4.55. However, when compared to withanolides devoid of 17α-OH, a down field shift of 0.15ppm is experienced by the 22-H signal of Dq-2. This shift is characteristic of 17α-hydroxy withanolides. The shift, therefore, fixes one α-OH at C-17 position of the molecule and this is also supported by MS.

The signals at $\delta$ 1.52s and 1.58s are attributed to protons of two methyl groups, 27 and 28 respectively. The NMR shows a significant difference in the positions of C-27 and C-28 proton signals, which have appeared very much up field, as compared to corresponding signals of the withanolides. This can be explained when, unlike the other withanolides, the stereochemistry of C-22 is put as S. In this case the plane of the lactone ring lies at about 90° with respect to C(20) - C(22) bond, with the result that 27 and 28 methyls "see" the rest of the molecule and in NMR, therefore, appear in the up field. This presumption is also based on the CD studies of daturalactone.

NMR suggests the following structure (I) for Dq-2. The proof for this structure is further substantiated by direct comparison of the oxidation product of daturalactone with Dq-2; they are identical with one another in all respects.
Mass spectrum (Fig. 3):— The molecular ion peak in the MS appeared at m/e 484. This supports the structure (I) and its microanalytical data. The base peak at m/e 125, prominent in all withanolides,
is formed by the cleavage of the C(20) - C(22) bond and is characteristic for such a lactone system. The peak at m/e 209 is characteristic for fragmentation induced by C-17 -OH. Fragmentation can be represented in a way similar to that proposed by Lavie for withanolides.

![Chemical structure diagram]

Hydrogenation (1 mole) of Dq-2 over Pd-C (10%) in EtOAc gave a crystalline compound m.p. 278 - 80°C.

Element analysis:

Found: C, 66.88; H, 7.72.

Calculated for C_{28}H_{38}O_{7}: C, 67.07; H, 7.81%.
Spectral data :-

UV spectrum:- UV showed $\lambda_{\text{MeOH}}^{\text{max}}$ at 227 nm and a low intensity band between 320-260 nm. There is a significant lowering in the intensity of the UV absorption band (227 nm) in the dihydro derivative. This shows that the double bond of the $\alpha, \beta$-unsaturated carbonyl chromophore of Dq-2 has only got hydrogenated.

IR spectrum (Fig.4):- IR spectrum of dihydro Dq-2 shows principal bands at 3472, 1737, 1700, 1400, 1137 and 985 cm$^{-1}$.

IR spectrum shows that the double bond of the $\alpha, \beta$-unsaturated ketone has got saturated in the hydrogenated product and has, therefore, got displaced to 1700 cm$^{-1}$.

Mass spectrum (Fig.5):- MS shows $M^+$ at 486 and other major fragments at m/e 471, 468, 450, 141, 125, 123, 121, 109, 105, 81, 79, 69, 67, 57, 55 etc.

CONCLUSION

Chemical, spectral data and identity with the oxidation product of daturalactone indicate that Dq-2 has got structure $I - 5\alpha, 17\alpha$-dihydroxy-1,12-dioxo-$6\alpha, 7\alpha$-epoxy-22S-witha-2,24-dienolide. This is the report of the isolation of second 12-oxowithanolid from natural sources. When the work was started and till the isolation and characterisation of the compound Dq-2, no 12-oxowithanolide was known.
Fig. 1 - IR (KBr) spectrum of Dq-2

Fig. 2 - NMR (100 MHz, CDCl₃) spectrum of Dq-2

Fig. 3 - MS of Dq-2
Fig. 4 - IR (mujol) spectrum of dihydro Dq-2

Fig. 5 - MS of dihydro Dq-2
Solubility:

It behaved like Dq-2 in solubility and thus falls in the group "N<sub>2</sub>" of compounds according to Shriner et al.<sup>35</sup>

Tests for elements and functional groups (chemical methods):

1. Detection of elements: It showed the absence of N, S and halogens in the Lassaigne's test.<sup>36</sup>

2. Groups found absent: The following functional groups tested in a way similar to Dq-2 were found absent:
   (i) Free -COOH group.
   (ii) Phenolic -OH group.
   (iii) Methylenedioxy group.
   (IV) Methoxyl group.
   (V) Primary and secondary alcoholic groups.

3. Groups found present: The compound indicated the presence of the following types of groups. Tests were performed in a way similar to Dq-2.
   (i) Epoxide group.
   (ii) Lieberman - Burchard test for steroids.

Spectral data:

UV spectrum: UV spectrum shows $\lambda_{\text{MeOH max}}$ at 225 nm. This shows the presence of an $\alpha,\beta$-unsaturated ketone or lactone chromophore or both, in the molecule.

IR spectrum (Fig.6): IR shows principal bands at 3438 (broad), 2970, 2940, 1730, 1710 (weak band), 1683, 1390, 1300, 1135 and 910 cm<sup>-1</sup>.
The broad band at 3438 cm\(^{-1}\) shows the presence of \(-\text{OH}\) groups. Since the compound remains unchanged upon attempted acetylation under mild conditions (\(\text{Ac}_2\text{O} + \text{pyridine}\)), this shows that the \(-\text{OH}\) groups are tertiary in nature. The band at 1683 cm\(^{-1}\) is due to \(\alpha,\beta\)-unsaturated ketone group and the one at 1730 cm\(^{-1}\) due to a six-membered-ring-\(\Delta\)-lactone with unsaturation \(\Delta^\to\) to \(>\text{C}=\text{O}\) group. The low intensity band at 1710 cm\(^{-1}\) can be attributed to a saturated six-membered ring ketone or to a Fermi resonance.

**NMR spectra (Fig. 7):** The NMR spectrum of Dq-3 is recorded in Table 2 along with proton assignments. Most of the signals of Dq-3 are similar to the corresponding signals of Dq-2. However, a significant difference between the positions of the C-18 proton signal and the up field shift of most of the other signals (from Dq-2 signals) is observed. The up field shift of 0.27ppm, experienced by the C-18 proton signal, as well as the slight up field shift of other proton signals in Dq-3 can be attributed to the absence of a 12-keto function in the molecule.

The position of the signals can be interpreted in a way similar to Dq-2. Thus a double doublet at \(\delta\) 5.8 (10;3;1) due to C-2 proton and a double triplet at \(\delta\) 6.54 (10;4.5;3) due to C-3 proton suggest an \(\alpha,\beta\)-unsaturated ketone, having \(>\text{C}=\text{O}\) at C-1, in the molecule. The two doublets at \(\delta\) 3.03 (4) and \(\delta\) 3.3 (4:1) due to 6- and 7-\(\beta\) protons, respectively, show the presence of an \(\Delta\)-epoxide at these positions. A multiplet at \(\delta\) 4.5 is due to C-22 proton and peaks at \(\delta\): 0.73s, 1.16s, 0.92d(7), 1.48s and 1.56s are attributed to C-18, C-19, C-21, C-27 and C-28 protons respectively. The peak at \(\delta\) 3.14s is due to an \(-\text{OH}\) group as it gets exchanged with D\(_2\)O.

**NMR suggests the following tentative structure II for Dq-3:**
However, this structure does not account for the presence of a saturated six-membered ring ketone function, which has been shown by IR as a weak intensity band at $1710\ \text{cm}^{-1}$ in the molecule.

Table 2

<table>
<thead>
<tr>
<th>$\delta$</th>
<th>No. of protons</th>
<th>multiplicity</th>
<th>proton assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.8</td>
<td>1</td>
<td>dd (10;3;1)</td>
<td>C - 2 (H)</td>
</tr>
<tr>
<td>6.54</td>
<td>1</td>
<td>dq (10;4.5;3)</td>
<td>C - 3 (H)</td>
</tr>
<tr>
<td>3.03</td>
<td>1</td>
<td>d (4)</td>
<td>C - 6 (H)</td>
</tr>
<tr>
<td>3.3</td>
<td>1</td>
<td>d (4;1)</td>
<td>C - 7 (H)</td>
</tr>
<tr>
<td>3.14</td>
<td>1</td>
<td>s</td>
<td>-OH</td>
</tr>
<tr>
<td>4.5</td>
<td>1</td>
<td>m</td>
<td>C - 22 (H)</td>
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<tr>
<td>0.73</td>
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<td>C - 18 (3H)</td>
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<tr>
<td>1.16</td>
<td>3</td>
<td>s</td>
<td>C - 19 (3H)</td>
</tr>
<tr>
<td>0.92</td>
<td>3</td>
<td>d (7)</td>
<td>C - 21 (3H)</td>
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<tr>
<td>1.48</td>
<td>3</td>
<td>s</td>
<td>C - 27 (3H)</td>
</tr>
<tr>
<td>1.56</td>
<td>3</td>
<td>s</td>
<td>C - 28 (3H)</td>
</tr>
</tbody>
</table>

$s = \text{singlet, } d = \text{doublet, } dq = \text{double quartet, } m = \text{multiplet. Coupling constants are in Hz and given in brackets.}$
MS spectrum (Fig. 8): MS shows $M^+$ at 470 and other fragments due to the loss of CH$_3$ and H$_2$O at m/e 455, 452 and 434. The spectrum is dominated by the cleavage of the C(20) - C(22) bond, common to all withanolides, giving rise to the base peak at m/e 125. The fragmentation induced by the hydroxyl group at C-17 position, with the cleavage of C(13) - C(17) and C(14) - C(15) bonds, gives rise to peak at m/e 209.

CONCLUSION

From the chemical analysis and spectral data the structure II - 5α,17α-dihydroxy-1-oxo-6α,7α-epoxy-22S-witha-2,24-dienolide, has been assigned to Dq-3. The compound, however, needs further chemical investigation in support of its formulation. The compound is a new withanolide.
Fig. 6 - IR (KBr) spectrum of Dq-3

Fig. 7 - NMR (100 MHz, CDCl₃) spectrum of Dq-3

Fig. 8 - MS of Dq-3
Solubility:

It behaved like Dq-2 in solubility and thus falls in "N" class of compounds according to Shriner et al.

Tests for elements and functional groups (chemical methods):

1. The compound showed the absence of N, S and halogens in the Lassaigne's test.

2. The compound showed the presence of the following groups:
   (i) It gave a pink-blue-green colour in Lieberman-Burchard test — It is a steroid.
   (ii) It contains a primary or a secondary -OH group.

Element analysis:

Found: C, 84.1; H, 11.87.
Calculated for C_{29}H_{50}O : C, 83.99; H, 12.15%.

Molecular formula suggests that the compound contains total five number of rings and double bonds.

Acetylation:

Upon acetylation (Ac_2O + pyridine) the compound formed a mono-acetate, m.p. 124 - 126°.

The element analysis, m.p., m.p. of acetate, mixed m.p. and co-TLC with an authentic sample of β-sitosterol suggested that the compound Dq-4 is β-sitosterol.
Dq-5

**Solubility** :-

The compound behaved like Dq-2 in solubility and, therefore, falls in "N" class of compounds according to Shriner et al.\(^{35}\)

**Element analysis** :-


Calculated for \(\text{C}_{16}\text{H}_{34}\text{O}\): C, 79.26; H, 14.14 %.

Molecular formula shows that the compound does not contain a multiple bond or a ring.

M.p. 49°, molecular formula, m.m.p. and co-TLC with an authentic sample of cetyl alcohol indicated that the Dq-5 compound is cetyl alcohol.
Fresh leaves of *Datura quercifolia* (2 kg), harvested in the middle of August, were cut into small pieces and extracted twice with 3L portions of cold benzene for 3 hr. The benzene extracts were mixed and concentrated to about 400ml. On keeping at 0° for 24 hr. a white crystalline solid of daturalactone (Dq-1) got deposited. The solution was filtered and vacuum dried. This gave a dark green gummy mass.

3g of this dark green gummy mass were dissolved in a little quantity of benzene-methanol mixture and the solution adsorbed over a small quantity of silica gel. This gel was charged on a column of silica gel (100g/100 - 200 mesh, 42cm x 3.2cm). Elution (solvent height 14cm) was started as follows and the various cuts monitored by TLC (for benzene fractions, benzene : EtOAc, 4:1; for other fractions benzene : EtOAc, 1:1):

<table>
<thead>
<tr>
<th>Fractions</th>
<th>1 - 32 benzene</th>
<th>32 x 25ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractions</td>
<td>33 - 56 benzene:EtOAc (8:1)</td>
<td>24 x 25ml</td>
</tr>
<tr>
<td>Fractions</td>
<td>57 - 71 benzene:EtOAc (8:2)</td>
<td>15 x 25ml</td>
</tr>
</tbody>
</table>

Mixed fractions 9 - 36 (530mg) and 46 - 70 (600mg) and concentrated them to dryness.

**Isolation of Dq-3.**

Dissolved the above fractions 46 - 70 in a small quantity of CHCl₃. Adsorbed this solution over a small amount of silica gel and charged this gel on a silica gel column (50g/100 - 200 mesh, 22cm x 2.6cm).
Elution (solvent height 18cm) was started as follows:

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Range</th>
<th>Solvent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractions</td>
<td>1 - 60</td>
<td>benzene</td>
<td>60 x 15ml</td>
</tr>
<tr>
<td>Fractions</td>
<td>61 - 100</td>
<td>benzene:EtOAc (20:1)</td>
<td>40 x 10ml</td>
</tr>
<tr>
<td>Fractions</td>
<td>101 - 123</td>
<td>benzene:EtOAc (20:2)</td>
<td>23 x 10ml</td>
</tr>
</tbody>
</table>

Fractions 72 - 97, which were found to be TLC pure (EtOAc:benzene, 1:1), were pooled and concentrated to dryness to give a white crystalline solid. This solid on crystallization from benzene-EtOAc gave white silky crystalline threads of Dq-3 (60mg), m.p. 281 - 282.5°C, Rf: 0.51 (EtOAc:benzene, 1:1), M⁺ 470.

Isolation of Dq-2.

Fractions 107 - 111 (isolation of Dq-3) showed a single spot on TLC (benzene:EtOAc, 1:1). These were pooled and the solution vacuum dried to give a white crystalline solid. This solid on crystallization from CHCl₃-light petrol gave white silky threads of Dq-2 (45mg), m.p. 303 - 305°C, Rf: 0.35 (EtOAc:benzene, 1:1), C₂₉H₂₆O₇, M⁺ 484 (Found: C, 67.1; H, 7.51. Calculated for C₂₉H₂₆O₇: C, 67.35; H, 7.43 %).

Hydrogenation of Dq-2:— Dq-2 (20mg) was hydrogenated over 10% Pd-C in EtOAc (hydrogen uptake 1 mole per mole of Dq-2). The mixture was filtered and the filtrate concentrated to dryness. Crystallization from EtOAc - benzene furnished shining microcrystalline needles of dihydro Dq-2 (16mg), m.p. 278 - 80°C, Rf: 0.45 (EtOAc:benzene, 1:1), C₂₈H₃₈O₇, M⁺ 486 (Found: C, 66.88; H, 7.72. Calculated for C₂₈H₃₈O₇: C, 67.07; H, 7.81%).

Oxidation of daturalactone (Dq-1):— Oxidation of daturalactone was carried out in a way similar to that described by Dhar et al. The
product was found to be identical in all respects with Dq-2.

Isolation of Dq-4 and Dq-5.

Fractions 9-36 (start of experimental) were dissolved in a small quantity of benzene. The mixture was mixed with about 4g of silica gel and this gel charged over a column of silica gel (40g/100 - 200 mesh, 29cm x 2cm) in light petrol. Elution (solvent height 21cm) was started as follows:

Fractions 1 - 20 light petrol 20 x 20ml
Fractions 21 - 36 light petrol:benzene(3:1) 16 x 20ml
Fractions 37 - 56 light petrol:benzene(1:1) 20 x 10ml
Fractions 57 - 95 benzene 28 x 10ml

Fractions 27-34 and 67-81 were pooled on the basis of TLC results (light petrol : EtOAc, 4:1 for fractions 27-34 and EtOAc : benzene, 1:1 for fractions 67-81).

Fractions 27-34 were evaporated to dryness under vacuum. An oily mass was obtained. On cooling at 0° the oil separated into a white solid. Crystallization from light petrol-EtOAc gave white flake like crystals of Dq-5, m.p. 49°, Rf: 0.78 (benzene : EtOAc, 3:2. M.m.p. and co-TLC with an authentic sample of cetyl alcohol confirmed the identity of the compound as cetyl alcohol.

Fractions 67-81 were vacuum dried. Crystallization from EtOAc-light petrol gave a silky crystalline solid of Dq-4, m.p. 136-37°, Rf: 0.78 (CHCl₃). M.m.p. and co-TLC with an authentic sample of β-sitosterol showed that Dq-4 was β-sitosterol.
β-sitosterol acetate:—  β-sitosterol was acetylated by keeping a mixture of β-sitosterol (20mg), acetic anhydride (0.5ml) and pyridine (1ml) at room temperature for 24 hr. The product was worked up as usual. Crystallization from benzene-light petrol furnished a white crystalline solid (14mg), m.p. 124–126°, Rf: 0.81 (benzene).
REFERENCES


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BOOKS USED FOR GENERAL REFERENCE