The Molecular Formula of Nimbin

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Mitra\(^1\) had suggested a molecular formula, \(C_{28}H_{54}O_8 (\ldots \text{CH}_3)\), for nimbin based on the Rast method of molecular weight determination. Narasimhan\(^2\) (who worked on this compound) later got more accurate molecular weight determinations by the X-ray method on (i) nimbin and (ii) one of its derivatives, "pyronimbic acid," and found that \(C_{30}H_{36}O_9\) represented the molecular formula of nimbin. Sen Gupta and co-workers, who later in a series of publications\(^3\) had repeated the earlier work\(^2\) and confirmed most of it, however, proposed a molecular formula \(C_{29}H_{35}O_9\) for nimbin based on the Rast method of molecular weight determination, and their analytical results.

To settle this point, we repeated the preparation of nimbin and its several derivatives including the ones described\(^1\)–\(^3\) and carefully analysed them. In our hands nimbin and its derivatives analyse correctly only for a molecular formula of \(C_{30}H_{36}O_9\) for the former. Quite a few of the analytical figures of Sen Gupta \textit{et al.} fit for the same as well, and some (e.g., their original analytical figures\(^6\) for the three isomeric decarboxy-nimbic acid esters) fit even better for \(C_{29}H_{35}O_9\). The question was finally settled by mass and N.M.R. spectral measurements on the compound. By the mass spectral method hexahydonimbin\(^*\) gives a molecular weight of 555±4 and the N.M.R. spectral results on nimbin and its derivatives show that nimbin contains 36 protons. These results would exclude the \(C_{29}H_{35}O_9\) formula leaving \(C_{30}H_{36}O_9\) as the correct molecular formula of nimbin.

We thank Dr. R. I. Reed and W. K. Reed of the University, Glasgow, for the mass spectral data, and Dr. A. Melara, Varian Associates, Switzerland, Dr. K. Nagarajan and Professor D. H. R. Barton for the N.M.R. Spectra.

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\(^3\) Sengupta, P., Sengupta, S. K. & Khastgir, H. N., \textit{(a) Chem. & Ind.}, 1958, 1402; \textit{(b) 1959, 397; (c) 1959, 1194; (d) Tetrahedron, 1960, 11, 67

\(^*\) The compound obtained on hydrogenation of nimbin in methanol over 5\% Pd/C under a pressure of 20 lbs./sq. in. and described before as "tetrahydro nimbin"\(^2\) is now found to be hexahydonimbin, wherein the ethylenic linkage conjugated with the ketone and the furan ring are saturated. We have also found by physical and chemical data that nimbin contains only two methoxy groups and nimbic acid contains none.
Structure of Nimbin

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Recent work on the structure and stereochemistry of bitter principles having a basic skeleton of 26 carbon atoms and a $\beta$-substituted furan ring has shown that they are invariably derivable from a triterpene precursor of the apoeuphol type.\textsuperscript{1,2} Nimbin, C$_{30}$H$_{48}$O$_{9}$,\textsuperscript{3} has in addition to a $\beta$-substituted furan ring ($\gamma$ 2-66, 2-75, 3-65) two carboxethoxy groups ($\gamma$ 6-27, 6-36), one acetate ($\gamma$ 7-97), a ketone conjugated with a $\text{cis}$ disubstituted double bond and having a tertiary $\gamma$-carbon atom (typical AB quartet centred at $\gamma$ 3-88, J 10 c.p.s.), a cyclic ether and an isolated double bond. Hence it is tricarbocyclic and has a basic skeleton of 26 carbon atoms. We propose the tentative structure (I) for nimbin which is derivable from apoeuphol by oxidative cleavage of ring C between C(12) and C(13) and appropriate oxidations at other sites and which is in complete accord with all available physical and chemical data.

"Pyronimbic acid," a neutral product,\textsuperscript{4} C$_{25}$H$_{28}$O$_{5}$, obtained by heating nimbinic acid (II) is now assigned complete structure (IV). N.M.R. comparison of "pyronimbic acid" with desacetyl nimbin (III) shows that at C(4) there is a methyl group, since the signal for a tertiary methyl group on a saturated carbon in the latter is shifted down to $\gamma$ 7-9 in the former. Dihydronimbin (II, A-2-double bond saturated) on treatment with acetic anhydride and pyridine gives a neutral compound, C$_{26}$H$_{28}$O$_{6}$ (Found: C, 71-6; H, 6-8, Calc.: C, 71-5; H, 6-5), m.p. 245-47°, $[\alpha]_{D}$ +60°. Its ultraviolet and infrared absorption spectra show the absence of ketone and hydroxyl groups, but reveal the presence of an enol-S-lactone (1754, 1645 cm.$^{-1}$) and a $\gamma$-lactone (1773 cm.$^{-1}$) formed by the lactonisation of the two carboxy groups with the enol of the C(1)-ketone and with the hydroxyl group. The C(4) carboxy group cannot form the enol-$\delta$-lactone with the C(1)-ketone as that will involve a double bond at the bridge-head. This fixes the position of the hydroxyl $\gamma$ to the C(4)-carboxyl and as the hydroxyl is secondary (oxidisable to a ketone) the acetate in nimbin is attached to C(6).

Placing of the methyl groups at C(8) and C(10) and the ether oxygen at C(7) in a five-membered ring is supported by the isolation of two products on dehydrogenation of the amorphous product obtained by lithium aluminium hydride reduction of nimbin, (i) 1,2,5-trimethyl naphthalene and (ii) a compound (Found: C, 85-9; H, 7-7, whose T.N.B. adduct (Found: C, 60-26; H, 4-8), m.p. 126°), b.p. < 100/1 mm. having ultraviolet spectra markedly resembling that of naphtho- (2,3-b) furan.\textsuperscript{4a} The $\beta$-substituted furan ring and the remaining six carbon atoms are assigned to ring D for the following reasons. Hexahydronimbin\textsuperscript{5} (wherein the $\Delta^2$-double bond and the furan are saturated) shows unsaturation to tetranitromethane, gives strong end absorption ($\lambda$ 208, $\varepsilon$ 8500) in the ultraviolet region and on permanganate-periodate, and iodine monobromide titrations shows the presence of one double bond. Its N.M.R. spectrum shows a methyl group on a double bond ($\gamma$ 8-3). The splitting of this methyl group into a doublet (J 1-5 c.p.s.), clearly visible in nimbin and many of its derivatives, we consider, is due to long range coupling with C(15)-H. The C(15)-H, besides being coupled to C(13)-methyl is also coupled to the adjacent methylene and shows up as a broad triplet at $\gamma$ 4-42 (J 6-5 c.p.s.) in nimbin. Though the position of this proton appears somewhat low, it is found that protons in analogous environments show similar chemical shifts. Isoneoheleneline, isoxomexicanin A, plumeracin and a rearrangement product of 5-nitrobornene are but a few examples. Hexahydronimbin on acid-catalysed isomerisation gives hexahydronimbin (Found: C, 65-7; H, 7-7), m.p. 202-3°, $[\alpha]_{D} + 18°$. Nimbin and dihydronimbin on similar isomerisation give isonimbin (Found: C, 66-8; H, 6-9.
Calc. C, 66.7; H, 6.7% m.p. 243–45°, and isodihydro-nimbin (Found: C, 66.2; H, 6.9% m.p. 200–202°, [α]D +90° respectively, both having a new chromophore, λmax. 233 μm, ε = 9000, which persists on lithium aluminium hydride reduction. Both these compounds give a reddish brown colour with tetranitromethane and their N.M.R. spectra show that two of the furan protons have shifted downfield showing thereby that the furan is now conjugated with a double bond. The double bond in (I) on acid treatment could move into conjugation with the furan ring to give (a) a 13,17-double bond with the (C(13))-methyl on it, or (b) a 16,17-double bond with a proton on C(13) causing the C(13)-methyl to appear as a doublet at higher fields in the N.M.R. or (c) a 16,17-double bond with an allylic migration of the ether from C(15) to C(13) as in (VI). Both (b) and (c) but not (a) will have an additional vinyl proton at C(16). The N.M.R. spectra of isonimbin and isodihydronimbin show the four methyl groups as sharp singlets at τ 8.23, 8.7, 8.73 and 9.05 and a new vinyl proton as a narrow triplet at τ 4.2. Hence isonimbin should be regarded as (VI).

As expected of this assignment the coupling constants of H3, H1, H8, H6, and H7 are identical with those in nimbin and the chemical shifts of these and those of the carboxyl groups and acetate are also not very different. The protons at C(9), C(11), C(14), and C(15) give a complex absorption of intensity six protons between 7 and 8 in isonimbin. The broad triplet at τ 4.42 assigned to the (C(15)) proton has disappeared from the spectra of these iso compounds.

Isodihydronimbin on selective hydrogenation with one mole of hydrogen gives isotetrahydronimbin, C36H46O9 (Found: C, 65.7; H, 7.8% m.p. 188–90°, [α]D +6500, and N.M.R. spectra show that the furan is no longer conjugated, the vinyl proton has disappeared but the methyl groups have the same chemical shifts as in isodihydronimbin. This is in complete accord with structure (VI) for the D-ring of the iso compounds.

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References
Stereochemistry of Nimbin

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In the N.M.R. spectra of nimbin (whose gross structure is proposed on biogenetic and other grounds as (I)* in the preceding communication) the C(6)-H appears as a quartet centred at 4.77 (J 3 c.p.s. and 12.5 c.p.s.). The assignment is based on the fact that in desacetyl nimbin this proton moves upfield (~ 2 ppm). Centred at 5.94 (J 3 c.p.s.) is one of the protons that couples with C(6)-H. It is assigned to C(7) since the chemical shift indicates a proton on a carbon having an oxygen function on it. This coupling pattern can then fit in only for a C(5)-C(6)-diaxial, C(6)-C(7)-axial equatorial disposition of the protons in a rigid six-membered ring. The C(5) proton appears as a doublet (J 12.5 c.p.s.) at 6.34 in nimbin, partially masked by the methoxyl peaks, but clearly visible in the spectra of dihydronimbin, or isonimbin (vide infra) or nimbin itself in pyridine solution. The doublet assigned to the C(5)-H (J 12.5 c.p.s.) is also visible in the spectra of "pyronimbic acid" (V), C_7H_30O_6, (Found: C, 71.6; H, 7.0. Calc.: C, 72.0; H, 6.1%). but in this case this doublet shows evidence of further coupling whereas the doublet assigned to C(7)-H (J 3 c.p.s.) is still sharp. This follows that the proton that is axially coupled to H(6) is allylic and axial to the 3,3-double bond and couples with H(3). This confirms the configurations assigned to the protons at C(5), C(6) and C(7) and shows besides, that the A and B rings are trans-locked, as shown in (I). The sharpness of the doublets of the C(5) and C(7) protons in nimbin indicates that C(4), C(10) and C(8) are tetrasubstituted. As expected of methyls at these positions, they appear as singlets at ~ 8.64 (6 protons), and 8.71 (3 protons) in nimbin. One of them shifts considerably down to ~ 8.4 in desacetyl nimbin. In "pyronimbic acid" (IV), this shift is to ~ 7.9 from 8.1 in its acetate (V), showing thereby that it is the C(4) methyl group that is involved in these shifts. Such deshielding of a methyl by a hydroxyl requires a 1,3-diaxial or equivalent disposition of the two groups and hence in the case of the C(6) equatorial hydroxyl group, it would require the C(4) methyl group also to be equatorial in nimbin as shown in (I).

The O.R.D. curve of hexahydronimbin is very similar to that of 1-ketocholestane and hence structure (I) would represent the absolute stereochemistry of nimbin.

We are indebted to Professor W. Klyne, Westfield College, London, Professor J. Levisalles and Miss Helene Hermann, University of Strasbourg, France for the O.R.D. curves and Dr. A. Melera, Varian Associates, Switzerland for some of the N.M.R. spectra.

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Since the submission of our manuscripts for publication a note by Henderson, R., McCrindle, R., Overton, K. H., Harris, M. & Turner, D. W. appeared in Proc. chem. Soc., 1963, 269, on "The Constitution of Nimbin." Their proposal for a partial structure of nimbin, viz., of rings A and B with the stereochemistry of ring B is in agreement with our proposal, but we differ for the environment proposed for the rest of the molecule.

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Nimbin, the main crystalline bitter constituent obtained from *Melia azadirachta* Linn., is proposed to have the structure and stereochemistry of a triterpenoid derivable from apoeuphol with the C-ring oxidatively broken between C11 and C13 and appropriate oxidations at other sites.

The molecular formula of nimbin has been in dispute and the formulæ $C_{30}H_{48}O_{9}$ ($\pm$CH$_2$) (ref. 1), $C_{28}H_{36}O_{9}$ (ref. 2) and $C_{28}H_{36}O_{9}$ (ref. 3) have been suggested for it. The molecular formula has been shown by us by careful elemental analyses of a number of nimbin derivatives and by mass and NMR spectral data as $C_{28}H_{36}O_{9}$. This has also been corroborated independently. The functional groups of nimbin have now been confirmed by NMR spectra and further chemical evidence. The two carbomethoxy groups ($\tau$ 6-27, 6-36), one acetate ($\tau$ 7-97) and the $\beta$-substituted furan ring ($\tau$ 2-66, 2-75, 3-65) are readily discernible in the spectra of nimbin and many of its derivatives. Of the two partial structures (I) and (II) proposed earlier, which would contain the $\alpha,\beta$-unsaturated ketone of nimbin, (I) with the cis-disubstituted double bond and the quaternary $\gamma$-carbon atom has been found to be the correct one by the typical AB quartet with doublets centred at $\tau$ 3-63 and 4-2 (1 10 cps) in the NMR spectra of nimbin, which disappears in that of dihydronimbin, wherein the conjugated double bond is saturated. Hexahydronimbin, obtained by the hydrogenation of nimbin over palladium-charcoal catalyst, is still unsaturated to tetranitromethane, and titrates with permanganate, periodate or iodine monobromide indicating the presence of one double bond. The NMR spectrum of nimbin also shows that it contains a cyclic ether (*vide infra*). Nimbin is, therefore, tricyclic and contains a basic skeleton of 26 carbon atoms. Recent studies on the structure and stereochemistry of bitter principles having a basic skeleton of 26 carbon atoms and a $\beta$-substituted furan ring, viz. limonin, obacunone, nomilin, gedunin, khivorin and cedrelone, have shown that they are invariably derivable from a triterpene precursor of the apoeuphol type (IV)$^{6,7}$. We wish to propose structure (VI) for nimbin, which is readily derivable from apoeuphol by oxidative cleavage of ring C between C11 and C13 and appropriate oxidations at other sites as indicated in (V), and which is in complete accord with all available physical and chemical data.

The presence of the $\alpha,\beta$-unsaturated ketone, derived from the ultraviolet and infrared spectral comparisons$^{8,9}$ of nimbin and dihydronimbin, has now been confirmed by the preparation of a monooxime from dihydronimbin, whose ultraviolet spectrum shows the absence of the R-band for a ketone. This proves that nimbin contains one and only one ketone and hence the 9th oxygen function has to be incorporated in an ether linkage. Part structure (I) can only be accommodated in ring A of the triterpenoid skeleton. The ready decarboxylation of nimbin on hot hydrolysis to give mixtures of $\alpha,\beta$- and $\beta,\gamma$-unsaturated ketones which does not take place in the case of dihydro- and hexahydro-nimbin, wherein this conjugated double bond is saturated, requires the placing of the carboxyl group at C4, vinylogously $\beta$ to the C1-ketone in nimbin. We have proved the decarboxylation by trapping carbon dioxide in barium hydroxide during the hot hydrolysis of nimbin. The alkyl substituent at C4 has now been shown to be a methyl group by comparing NMR spectra of pyronimbic acid (I) (obtained by heating nimbic acid, VII) with that of desacetyl nimbin (VIII) since a methyl group on a quaternary C atom in the latter is shifted down to $\tau$ 7-9 in the former.

Dihydronimbinic acid (VII, $\Delta^2$-double bond saturated) on treatment with acetic anhydride and pyridine gives a neutral product, $C_{28}H_{36}O_{9}$. Its ultraviolet and infrared spectra show the absence of ketonic and hydroxyl groups, but reveal the presence of an enol-$\delta$-lactone (1754, 1645 cm.$^{-1}$) and a $\gamma$-lactone (1773 cm.$^{-1}$) formed by the lactonization of the two carboxyl groups with the enol of the C4-ketone and with the hydroxyl formed by the hydrolysis of the original acetate. Hexahydronimbinic acid obtained by the hydrolysis of hexahydronimbin also gives a similar product on the same treatment. The C4-carboxyl group cannot
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Form the enol-$\delta$-lactone with the $C_4$-ketone as that will involve a double bond at the bridgehead. This fixes the position of the hydroxyl group $\gamma$ to the $C_4$-carbonyl and as the hydroxyl is secondary (oxidizable to a ketone)$^9$ the acetate in nimbin is attached to the $C_4$-carbon atom.

The $C_6$-H appears as a quartet centred at $\tau$ 4.77 (1.3 cps and 12.5 cps) in the NMR spectra of nimbin. The assignment is based on the fact that in desacetyl nimbin this proton moves upfield (~1.5 to 2 $\tau$ units). Centred at $\tau$ 5.94 (1.3 cps) is one of the protons that couples with $C_6$-H. It is assigned to $C_7$ since the chemical shift indicates a proton on a carbon having an oxygen function on it. This coupling pattern can then fit in only for a $C_3$, $C_5$-di axial, $C_6$, $C_7$-axial equatorial disposition of the protons in a rigid six-membered ring (VI). The acetic acid side chain from $C_9$ needed for the formation of the enol-$\delta$-lactone with the $C_4$-ketone in these cases can readily be accommodated only with the cleavage of ring $C$ between $C_{12}$ and $C_{13}$ in the triterpenoid skeleton. The dilactones could thus be assigned structure (IX) and pyronimic acid structure (X). The $C_9$-proton appears as a doublet (1 12.5 cps) at $\tau$ 6.34 in nimbin partially masked by the methoxyl peaks, but clearly visible in the spectra of dihydronimbin or isonimbin (vide infra) or that of nimbin itself in pyridine solution. The doublet assigned to $C_9$-H is also visible in the spectra of pyronimic acid acetate (XI), but in this case, this doublet shows evidence of further coupling, whereas the doublet assigned to $C_7$-H (1 3 cps) is still sharp. It follows that the proton which is axially coupled to $H_6$ is allylic and axial to the $\Delta^5$-double bond and couples with $H_8$ (ref. 9, 10). This confirms the configurations assigned to the protons at $C_8$, $C_9$ and $C_{13}$ as shown in (VI) and shows besides that $A/B$ rings are trans-fused.

The sharpness of the doublets of the $C_9$ and $C_8$ protons in nimbin indicates that the $C_4$, $C_8$ and $C_9$ carbon atoms are quaternary. The methyl groups at these positions appear as singlets at $\tau$ 8-64 (6 protons) and 8-71 (3 protons) in nimbin consistent with their environments. One of these shifts considerably down to $\tau$ 8-4 in desacetyl nimbin. Similar shifts also occur in desacetyl dihydro- and desacetyl hexahydro-nimbins, compared with their acetates. In "pyronimic acid" this shift is to $\tau$ 7-9 from 8-1 in its acetate, showing thereby that it is the $C_4$-methyl group that is involved in these shifts. Such a deshielding of the methyl group by a hydroxyl group requires a 1,3 diaxial or equivalent disposition of the two groups$^{11}$ and hence in the case of the $C_6$-equatorial hydroxyl group it would require the $C_4$-methyl group also to be equatorial$^9$ as shown in (VI).

The placing of the methyl groups at $C_8$ and $C_{10}$ and the ether oxygen at $C_7$ in a five-membered ring is supported by the isolation of two products on dehydrogenation of the amorphous product obtained by lithium aluminium hydride reduction of nimbin. Such a deformylation product 1,3,itaclcarboxyl group on a double bond (7-8-3). The splitting of this methyl group into a doublet (1 1.5 cps) clearly visible in the spectra of nimbin and many of its derivatives, we consider, is due to long-range coupling$^{14,15}$ with $C_9$-H. The $C_9$-H, besides being coupled with the $C_{13}$-methyl, is also coupled to the adjacent methylene and shows up as a broad triplet at $\tau$ 4.42 (1 6.5 cps) in nimbin.

Though the position of this proton appears somewhat low it is found that protons in analogous environments show similar chemical shifts. Iso-eneholinol$^{18}$, isomexicanin$^{16}$, plumeracinn$^{17}$ and a rearrangement product of 5-nitrobornene$^{18}$ are but a few examples.

Hexahydronymbin, on acid catalysed isomerization, gives isohexahydronymbin which shows no strong absorption in the ultraviolet (A 208, $\epsilon 8500$) which is consistent with its formulation as a tetrasubstituted double bond$^{19}$. Its NMR spectrum shows a methyl group on a double bond (7-8-3). The ultraviolet spectra markedly resembling that of naphto-(2,3-b) furan$^{12}$. The 5-substituted furan ring and the remaining six carbon atoms are assigned to ring $D$ to accommodate the following facts. Hexahydronymbin gives strong end absorption in the ultraviolet (A 208, $\epsilon 8500$) which is consistent with its formulation as a tetrasubstituted double bond$^{19}$. Its NMR spectrum shows a methyl group on a double bond (7-8-3). The splitting of this methyl group into a doublet (1 1.5 cps) clearly visible in the spectra of nimbin and many of its derivatives, we consider, is due to long-range coupling$^{14,15}$ with $C_9$-H. The $C_9$-H, besides being coupled with the $C_{13}$-methyl, is also coupled to the adjacent methylene and shows up as a broad triplet at $\tau$ 4.42 (1 6.5 cps) in nimbin.

Though the position of this proton appears somewhat low it is found that protons in analogous environments show similar chemical shifts. Iso-eneholinol$^{18}$, isomexicanin$^{16}$, plumeracinn$^{17}$ and a rearrangement product of 5-nitrobornene$^{18}$ are but a few examples.

Hexahydronymbin, on acid catalysed isomerization, gives isohexahydronymbin which shows no strong absorption in the ultraviolet (A 210 $\mu$). Nimbin and dihydronimbin, on the other hand, on similar isomerization give isonimbin and isodiodyronymbin respectively, both having a new chromophore, $\lambda_{max}$ 233 $\mu$., $\epsilon$ 9000, which persists on lithium aluminium hydride reduction. Both these compounds give a reddish brown colour with tetraniitromethane and their NMR spectra show that two of the furan protons have

*One of us (N.S.N.) differs on this.
shifted downfield, both typical of the furan being conjugated with a double bond. The double bond in (VI) on acid treatment could move into conjugation with the furan ring to give (a) a 13,17-double bond with the C_{13}-methyl on it, or (b) a 16,17-double bond with a proton on C_{13}, causing the C_{13}-methyl to appear as a doublet at higher fields in the NMR, or (c) a 16,17-double bond with an allylic migration of the ether from C_{16} to C_{13} as in (XII). Both (b) and (c) but (a) will have an additional vinyl proton (C_{16}). The NMR spectra of isonimbin and isodihydronimbin show the four methyl groups as sharp singlets at \( \tau = 8.35, 8.7, 8.73 \) and 9.05 and a new vinyl proton as a narrow triplet at \( \tau = 4.2 \). Hence isonimbin should be regarded as (XII). As expected of this assignment the coupling constants of H_{9}, H_{5}, H_{6}, H_{7} and H_{8} are identical with those of nimbin and the chemical shifts of these and those of the carbomethoxy groups and acetate are not very different. The protons at C_{9}, C_{11}, C_{14} and C_{16} give a complex absorption of intensity 6 protons between 7 and 8 \( \tau \) in isonimbin. The broad triplet at \( \tau = 4.42 \) assigned to the C_{15} proton has disappeared from the spectra of these compounds. Isohydronimbin on selective hydrogenation with one mole of hydrogen gives isotoehydronimbin whose ultraviolet and NMR spectra show that the furan is no more conjugated, the vinyl proton has disappeared but the methyl groups have the same chemical shifts as in isodihydronimbin. Isohexahydronimbin also on hydrogenation gives isotoehydronimbin in which the vinyl proton has disappeared, but the methyl groups have the same chemical shifts as the parent compound. This is in complete accord with structure (XII) for the D-ring of the iso-compounds.

The easy hydrolysis of the ester of the tertiary carbonyl group at C_{4} must be due to participation by the hydrated form of the C_{4}-ketone or the C_{6}-hydroxyl group during hydrolysis. The difficulty to form derivatives of the ketone has to be attributed to steric hindrance due to the C_{15} carbomethoxy group. Absence of reactivity for the \( \alpha \)-ketomethyleneproton at C_{6} in the hydrogenated derivatives of nimbin in alkaline solution is explicable on the non-enzonilization of the C_{6}-ketone as it could stay as a lactol under the conditions with the hydrolysed C_{6}-carboxyl group.

The optical rotatory dispersion curve of hexahydronimbin is very similar to that of \( \beta \)-cholestan-3-one and hence structure (VI) would represent the absolute configuration of nimbin. Biogenetically nimbin is the first of the C_{29}terpenoid bitter principles to have the C-ring oxidized and broken.*

**Experimental Procedure**

Melting points were taken on a hot stage. Specific rotations were taken in chloroform solution at a concentration of 10 mg/ml at room temperature unless otherwise stated. Infrared spectra were taken in nujol mull on a Perkin-Elmer Infracord spectrophotometer. Ultraviolet spectra were taken in 95 per cent ethanol solution on a Unicam or Perkin-Elmer (350) spectrophotometer. NMR spectra were taken on a Varian A-60 spectrometer in CDCl_{3} solution unless otherwise stated. Brockman-standardized alumina was used for chromatography. The solvents used were of AR grade.

Nimbin used in these experiments was purified by chromatography on grade II alumina eluting with petroleum ether, petroleum ether-benzene, benzene and benzene-ether mixtures. Pure nimbin was eluted with 75 per cent ether in benzene. The analyses of nimbin and its derivatives to distinguish between \( C_{29}H_{36}O_{5} \) and \( C_{29}H_{36}O_{6} \) formulae are given in Table 1.

Nimbin gave a methoxyl value of 11.4 per cent (required for two, 11.5 per cent) and dihydronimbin gave 11.6 per cent (required for two, 11.5 per cent). Nimbic acid and dihydronimic acid gave no O-methyl value. Desacetyl nimbin gave a C-methyl value of 10.1 per cent (three C-methyl require 9.01 per cent and four, 12.2 per cent) and dihydro nimbic acid 12.2 per cent (four C-methyls require 12.7 per cent).

**Hexahydronimbin** — Five per cent Pd charcoal (1 g) was reduced in acetic acid (AR, 20 ml) and to this was added pure nimbin (500 mg) dissolved in acetic acid (AR, 40 ml) and hydrogenated at room temperature. Hydrogen uptake was 1 mole (7 min), 2 moles (18 min), and 3 moles (41 min). There was practically very little absorption afterwards. Hydrogenation was stopped after 80 min., catalyst filtered off, filtrate brought to a small volume and worked up in the usual manner. Crystallization from acetone-hexane gave hexahydronimbin (470 mg); m.p. 197.9°. A number of crystallizations raised the melting point to 198-200°. Chromatography of the crude product gave no fraction melting below 195.8°. Ultraviolet \( \lambda_{max} \) 290 nm (e 477), 208 nm (e 8500). Infrared and NMR spectra of hexahydronimbin showed that the furan ring is saturated (Found: C, 65-80; H, 7.50. \( C_{29}H_{40}O_{5} \) requires C, 65-95; H, 7.69%). Dihydro and hexahydronimbin and their acids did not give the Zimmermann test.

**Nimbinic acid and desacetyl nimbin** — These were also obtained by the mild hydrolysis of nimbin. (a) Nimbin (2.7 g) dissolved in benzene (AR, 40 ml) was added to 0.5 per cent methanolic potassium hydroxide solution (40 ml). After every 30 min., a 20 ml aliquot was worked up for neutral and acidic products. The neutral material was found to be desacetyl nimbin and the acid to be nimbinic acid. The relative proportions of the two were found to vary with time; after 30 min. the mixture gave 80 per cent desacetyl nimbin (1) and 12 per cent nimbinic acid (2), and after 120 min. 62 per cent of (1) and 38 per cent of (2).

(b) To nimbin (540 mg) in a mixture of benzene (4-5 ml) and methanol (13-5 ml), water (0.3 ml) and 10 per cent sodium hydroxide (0.3 ml) were added at 0° and the reaction mixture kept at room temperature for 4 hr. On working up, it gave
Table 1 — Analyses of Nimbin and Its Derivatives

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<td>Nimbin</td>
<td>66.94</td>
</tr>
<tr>
<td></td>
<td>6.84</td>
</tr>
<tr>
<td></td>
<td>C_2H_5O_2 requires C, 66.65; H, 6.70 and 67.04; H, 6.37</td>
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<tr>
<td></td>
<td>66.85</td>
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<tr>
<td></td>
<td>6.50</td>
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<td></td>
<td>C_2H_5O_2 requires C, 65.89; H, 6.87%</td>
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<tr>
<td></td>
<td>66.97</td>
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<tr>
<td></td>
<td>6.54</td>
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<tr>
<td>Dihydrornimbin</td>
<td>66.67</td>
</tr>
<tr>
<td></td>
<td>6.78</td>
</tr>
<tr>
<td></td>
<td>C_2H_5O_2 requires C, 66.40; H, 7.06 and 67.04; H, 6.72%</td>
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<tr>
<td></td>
<td>66.34</td>
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<td></td>
<td>6.69</td>
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<td>C_2H_5O_2 requires C, 65.64; H, 7.22%</td>
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<tr>
<td>Desacetyl nimbin</td>
<td>67.44</td>
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<td></td>
<td>6.59</td>
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<td></td>
<td>C_2H_5O_2 requires C, 67.45; H, 6.87 and 67.04; H, 6.72%</td>
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<tr>
<td></td>
<td>66.37</td>
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<td></td>
<td>6.43</td>
</tr>
<tr>
<td></td>
<td>C_2H_5O_2 requires C, 65.64; H, 6.60%</td>
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<tr>
<td>Nimbinic acid</td>
<td>66.6</td>
</tr>
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<td></td>
<td>6.8</td>
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<td>C_2H_5O_2 requires C, 66.08; H, 6.83 and 67.04; H, 6.72%</td>
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<tr>
<td></td>
<td>65.20</td>
</tr>
<tr>
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<td>7.00%</td>
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<tr>
<td>Dihydrornimbinic acid</td>
<td>65.7</td>
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<td></td>
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<td>C_2H_5O_2 requires C, 65.20; H, 6.83 and 67.04; H, 6.72%</td>
</tr>
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</table>

Neutral product (410 mg.; m.p. 211-13°) and the acid (98 mg.; m.p. 230-5°). Both desacetyl nimbin and nimbinic acid were recrystallized to correct melting points.

Increasing the quantity of water to 0.75 ml. and sodium hydroxide solution to 1.5 ml. in the above experiment gave 69 mg. of desacetyl nimbin (m.p. 215°) and 462 mg. of nimbinic acid (m.p. 243-6°). (c) To a solution of potassium bicarbonate (500 mg.) in a mixture of ethanol (47.5 ml.) and water (2.5 ml.) was added nimbin (530 mg.) and the mixture refluxed for 3 hr. Methanol was removed in vacuo, the mixture diluted with water, extracted with benzene for neutral material, and the aqueous solution acidified. Nimbinic acid which separated on acidification of the solution was extracted with chloroform and crystallized from aqueous methanol; yield 350 mg.; m.p. 256-8°.

Oxidation of desacetyl nimbin — To a solution of desacetyl nimbin (730 mg.) in dry pyridine (10 ml.) was added a solution of chromium trioxide (1.1 g.) in dry pyridine (45 ml.). After 3 days the mixture was diluted with water, extracted with ether and worked up. The product was crystallized from ether; yield 60 mg.; m.p. 176°, \( \lambda_{\text{max}} = 307 \times (e 440) \) (Found: C, 68.34; H, 6.0. C_2H_5O_2 requires C, 67.73; H, 6.5%). This experiment, however, often failed on repetition.

Nimbinic acid (100 mg.) was dissolved in 1N sodium hydroxide (2 ml.). Careful acidification of the solution with dil. hydrochloric acid gave nimbinic acid which was extracted with ether and crystallized from aqueous methanol; identified by m.p. and m.m.p.

Nimbinic acid (470 mg.) was dissolved in 5 ml. of 1N sodium hydroxide. To this and to an equivalent blank of 1N sodium hydroxide an excess of barium chloride solution was added. After heating on the steam bath for 2 hr the apparent equivalent weight was determined by back titration with standard acid. This was found to be 160 indicating either a tricarboxylic acid or a monocarboxylic acid plus one mole of carbon dioxide trapped as barium carbonate. The inorganic precipitate was filtered and identified as barium carbonate. The organic acid that precipitated out on acidification of the filtrate titrated as a monobasic acid of equivalent weight 420.

Dihydrornimbinic acid (500 mg.) dissolved in pyridine (2.5 ml.) and acetic anhydride (2.5 ml.) was heated on the steam bath for 2 hr. After working up, the neutral product was crystallized from acetone-ether (yield 100 mg.; m.p. 247-9°; \( \alpha_d +60° \)). No absorption in the ultraviolet at 290 mg or in the infrared in the hydroxyl region (Found: C, 71.6; H, 6.8. C_2H_5O_2 requires C, 71.54; H, 6.47%).

Hexahydrornimbinic acid was obtained by the cold hydrolysis of hexahydrornimbin and crystallized from ether had m.p. 230-5° (Found: C, 65.54; H, 7.65. C_2H_5O_2 requires C, 65.53; H, 7.4%). Hexahydrornimbinic acid on treatment with acetic anhydride-pyridine, as in the case of dihydrornimbinic acid, gave a neutral product; m.p. 255-7°; \( \alpha_d -21° \); infrared spectrum showed bands at 1786, 1754 and 1642 cm.\(^{-1}\). No absorption in the —OH region. No absorption around 290 mg in the ultraviolet (Found: C, 71.27; H, 7.37. C_2H_5O_2 requires C, 70.89; H, 7.32%).

Hexahydrodesacetyl nimbin was obtained by esterifying hexahydrornimbinic acid with diazomethane and crystallization from acetone-hexane; m.p. 160-2°; \( \alpha_d +92° \) (hygroscopic). The same compound was obtained by hydrogenating desacetyl nimbin over Pd-C under pressure as described for hexahydrornimbin from nimbin (Found: C, 66.5; H, 7.8. C_2H_5O_2 requires C, 66.6; H, 8.0%).

Estimation of double bonds in hexahydrornimbin by permanganate-periodate titration\(^{19}\) — The stock oxidant solution consisted of 20.86 g. (97.5 mmoles) of sodium metaperiodate and 250 ml. of 0.01M (2.5 mmoles) of potassium permanganate.

Hexahydrornimbin, camphene, santa-4-ene-olide (contains one double bond) and santanolide-a (saturated), 0.125 mmole each, were dissolved in 4 ml. of pure tert-butanol, 1 ml. of potassium carbonate solution (0.25 per cent), 3 ml. of water and 2 ml. of oxidant were added to each and kept at room temperature with a corresponding blank. Portions (1 ml.) were withdrawn at intervals and known volume of excess 0.01N sodium arsenite solution added and excess arsenite titrated against 0.005N iodine solution. It was found that after 72 hr, hexahydrornimbin and camphene titrated for 0.95 double bond each, santa-4-ene-olide for 1.1 double bond and santanolide-a for 0.05 double bond.

Titration for double bonds with iodine monobromide\(^*\) — Hexahydrornimbin, cholesterol acetate and camphor (0.1 mmole each) were taken in 5 ml. of carbon tetrachloride and 25 ml. of iodine monobromide solution added to each and kept for 1 hr in the steam bath.
dark along with a blank. After 1 hr, excess potassium iodide was added and titration of the excess iodine monobromide against 0.1N sodium thiosulphate solution showed the following values for the number of double bond in each compound: cholesterol acetate 0-9, camphor 0-0, hexahydrinbin 0-5. Repetitions with longer reaction time gave only similar results.

With peracids – Cholesterol acetate and hexahydrinbin (0.1 mmole each) were dissolved in 10 ml. of chloroform and to each were added 2 ml. of perbenzoic acid solution. An equivalent blank was also kept. The consumption of peracid was measured at intervals by titration of excess peracid left behind against N/100 thiosulphate solution and the number of double bonds in each compound calculated. Cholesterol acetate consumed peracid equivalent to one double bond after 5 hr and remained constant till 96 hr. Hexahydrinbin did not consume any peracid.

**Acetate of pyronimbic acid** – Pyronimnic acid (180 mg) in pyridine (1 ml) and acetic anhydride (1 ml) was kept at room temperature overnight. After working up it was crystallized from chloroform-methanol; m.p. 294-6° (decomp.); yield 113 mg. \( (x)_1 + 235° \), infrared spectrum showed bands at 1739, 1724, 1666 and 1250 cm.\(^{-1}\) (Found: C, 71-6; H, 7-0). NMR spectrum in CDC\(_1\)\(_2\) showed the presence of one carbomethoxy group and one acetate. On addition of trifluoroacetic acid to the solution the signal for the acidic proton was shifted downfield from 505 cps but \( \Delta \nu = -110° \). NMR spectrum in CDC\(_1\)\(_2\) requires C, 71-9; H, 7-7°o). Its infrared spectrum showed peaks at 3380, 1711, 1683 and 1620 cm.\(^{-1}\) (Found: C, 66-84; H, 7-02; C\(_9\)H\(_{10}\)O\(_9\) requires C, 71-9; H, 7-7°o). Its ultraviolet spectrum showed only low end absorption below 210 mu.

**Acid treatment of nimbinic acid** – Nimbinic acid (446 mg) was refluxed for 2 hr with methanol (17 ml) containing conc. HCl (3 ml.). On working up, it gave a dark neutral gum which crystallized from methanol to give a highly crystalline material which gradually charred above 300° (Found: C, 69-41; H, 6-33. C\(_{27}\)H\(_{30}\)O\(_9\) requires C, 69-51; H, 6-48°o). Its infrared spectrum showed peaks at 3380, 1711, 1683 and 1620 cm.\(^{-1}\), the last one being very intense.

**Sodium borohydride reduction of nimbin** – To a solution of nimbin (500 mg) in 80 per cent aqueous dioxane (27.5 ml) was added slowly with stirring to the course of 2-5 hr a solution of sodium borohydride (500 mg) in aqueous dioxane (22.5 ml). After allowing the reaction mixture to stand at room temperature for 22 hr, it was acidified to congo red with 2N sulphuric acid, extracted with chloroform and chromatographed on alumina. It was crystallized from ether; m.p. 195-6°. Its infrared spectrum showed hydroxyl groups (Found: C, 68-03; H, 8-08; OMe, 7-0; OAc, 9-31°o). Isonimbin – Nimbin (500 mg) in glacial acetic acid (10 ml) and hydrochloric acid (AR, 5 ml) was heated at 100° for 3 hr. The mixture was then poured into water and extracted with chloroform and worked up as usual. Chromatography over alumina in benzene gave initial fractions (100 mg) which on crystallization from ether had m.p. 247-8° (Found: C, 66-84; H, 7-02. C\(_{27}\)H\(_{30}\)O\(_9\) requires C, 66-65; H, 6-71°o). Its infrared spectrum showed no significant difference from nimbin. Ultraviolet absorption was identical with that of nimbin (500 mg) in benzene giving maxima at 250, 270, 310 and 340 mu.

We thank Shri N. K. Venkatasubramanian for doing this experiment.
traction curve, isonimbin minus nimbin, gives $\lambda_{\text{max}}$ 233 μm ($\epsilon$ 9315). After LiAlH$_4$ reduction in the usual way the compound still gave the ultraviolet absorption at 230 μm.

Isodihydronimbin was prepared as above from dihydronimbin and crystallized from ether; m.p. 203-5°; $\lambda_{\text{max}}$ 233 μm ($\epsilon$ 9000); (α)$_D$ +90° (Found: C, 66-2; H, 6-9. C$_{30}$H$_{40}$O$_9$ requires C, 66-4; H, 7-1%). Isonimbin and isodihydronimbin gave a reddish brown colour with tetranitromethane and the NMR spectra showed that an α-proton and the β-proton are shifted downfield compared to the parent compounds.

Dihydroisodihydronimbin — Isodihydronimbin (136 mg) was hydrogenated in ethyl acetate over pre-reduced 5 per cent Pd-C (400 mg) till one mole of hydrogen was absorbed. Crystallized from acetone-hexane; m.p. 195-7°; $\lambda_{\text{max}}$ 210 μm ($\epsilon$ 6500), 290 μm ($\epsilon$ 73) (Found: C, 66-1; H, 7-4%).

Acknowledgement

We wish to express our thanks to Prof. W. Klyne, Westfield College, London, Prof. J. Levisalles and Miss Helene Herrmann, University of Strasbourg, France, for the ORD curves and Dr A. Melera, Varian Associates, Switzerland, Dr K. Nagarajan, CIBA Research Centre, Bombay, Dr U. K. Pandit and Mr P. K. Krover, University of Amsterdam, for some of the NMR spectra and Prof. K. Venkataraman, Director, National Chemical Laboratory, for his interest in this work.

References