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# TETRANORTRITERPENOIDS—VIII[1]

## THE CONSTITUTION AND STEREOCHEMISTRY OF NIMBIN

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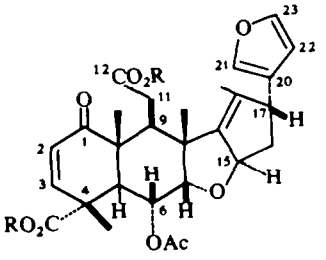
**Abstract**—The recorded evidence[3,4] bearing on the constitution of nimbin has been evaluated. By using spectroscopic and additional chemical evidence and making certain biogenetic assumptions, the constitution and stereochemistry (I) are deduced for nimbin. Pyronimbic acid[3] is formulated as XI. The dilactone XVII holds an important place in the structural argument.

NIMBIN was first isolated[2] in 1942 from the seed oil of *Melia azadirachta* and later from other parts of the same tree by Siddiqui *et al.*. Its chemistry subsequently attracted the attention of several Indian groups, and when we first considered the problem of nimbin in 1962, two publications[3,4] had appeared which provided a substantial basis for its solution. Moreover, the correct molecular formula, $C_{30}H_{36}O_9$, had then been established by mass-spectrometry.[5]

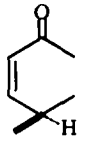
Two reactions in the published work were of signal importance. When nimbin is heated under reflux with potassium hydroxide in methanol, there result three isomeric acids, $C_{25}H_{30}O_6$.[4] These were chromatographically separated as the methyl esters and formulated[4] as the part structures II, III and IV, by Sengupta's group who correctly concluded that the acids each derive from nimbin by hydrolysis of one acetate and two methyl ester groups and loss of carbon dioxide. Sengupta also reinvestigated[4] Narasimhan's pyronimbic acid,[3] correctly formulated its chromophore as V or VI and by a combination of this evidence suggested for nimbin the part-structures VII or VIII. Spectroscopic evidence indicated the presence of a β-substituted furan ring,[3,4] thus accounting for eight of the nine oxygen atoms in nimbin.

We were able at the outset to confirm the previously suggested oxygen functions by examining the NMR spectra of nimbin and its derivatives. This, as will become apparent, also revealed the missing oxygen atom as a cyclic disecondary ether.

Reference to Fig. 1 readily confirms the β-substituted furan ring, two methyl esters and one acetate. The $\Delta^{\alpha\beta}$-γ-methoxycarbonyl ketone contained in Sengupta's part-structure VII or VIII was also convincingly evident. Thus both in nimbin (I) and the corresponding diethyl ester (IX), $C_{32}H_{40}O_9$, m.p. 174–176°, $[\alpha]_D$ +106°, H-2 and H-3 formed a typical AB quartet (τ 3·62, 4·10; $J_{2,3}$ = 10 Hz) and were not further coupled. However, in the decarboxylation product (X), obtained by alkaline hydrolysis of nimbin, and examined as the methyl ester acetate (Xa), $C_{27}H_{32}O_7$, m.p. 178–180°, $[\alpha]_D$ +178°, the vinyl protons, while still forming an AB quartet (τ 3·52,



I: R = Me
IX: R = Et
XV: 1,6-diketone
R = Me



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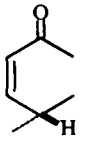

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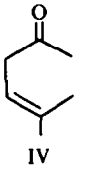

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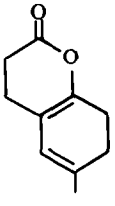
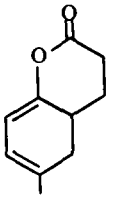
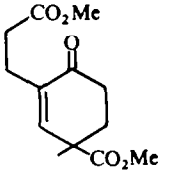


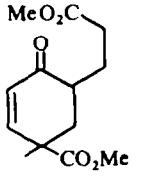
V



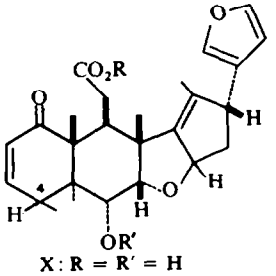
VI



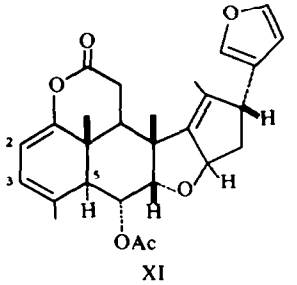
VII



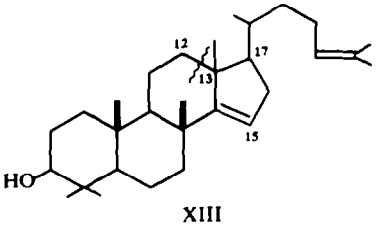
VIII



X: R = R′ = H
Xa: R = Me; R′ = Ac



XI

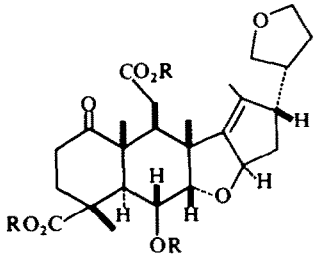


XIII

4·20), were both further coupled to the new proton at C-4 which, in turn, coupled with the new secondary Me group (τ 8·73).

The part of pyronimbic acid (XI) germane to the derivation of Sengupta's part-structure (VIII) for nimbin was also well supported by the NMR spectrum (which disallows the alternative VII). Thus the homo-annular diene in pyronimbic acid [$\lambda_{max}$ 280 nm; $\nu_{max}$ ($CHCl_3$) 1747 $cm^{-1}$], shows an AB quartet arising from H-2 and H-3, consisting of one sharp doublet (H-2; τ 4·49, $J_{2,3}$ = 7 Hz) and a multiplet (H-3; τ 4·20), attributable to coupling with H-2. H-5 (τ 7·12) and also the vinyl Me group at C-4 (τ 7·83). C-10 therefore does not bear hydrogen.

Nimbin must also have an isolated olefinic linkage since this survives in hexahydronimbin (XII), $C_{30}H_{42}O_9$, m.p. 204–206° (described by Narasimhan[3] as tetrahydronimbin), 3H signal at τ 8·2 (*d*) (vinyl methyl), $\lambda_{max}$ 213 nm (log ε 3·69).

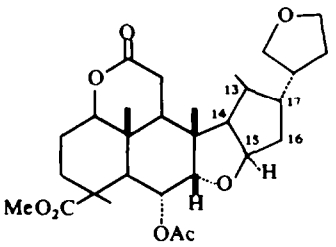



XII: R = Me
R′ = Ac
XVI: R = R′ = H

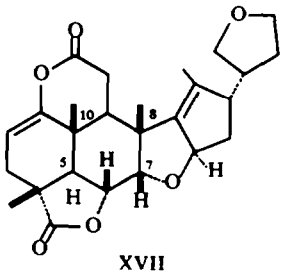
The above functional groups taken in conjunction with the molecular formula, $C_{30}H_{36}O_9$, demand that nimbin be tricarbocyclic. If one additionally considers that it has a basic $C_{26}$-carbon skeleton, contains a β-substituted furan ring and five C-Me groups (three tertiary, one vinyl and one oxidized to carboxyl) then a relationship to limonin,[6] and therefore derivation from apoeuphol (XIII) becomes an attractive probability. The "Sengupta chromophore" (VIII) can be readily accommodated by placing the CO group at C-1 and cleaving ring C of euphol between C-12 and C-13. C-12 then furnishes the enol-lactonic CO group of pyronimbic acid (XI).

The C-1/C-12 functionalities are further inter-related in the saturated δ-lactone (XIV), $C_{29}H_{42}O_8$, m.p. 269–271°, $[\alpha]_D$ ±0°, $\nu_{max}$ ($CCl_4$) 1755 (δ-lactone), 1738 (acetate and methyl ester) $cm^{-1}$, formed when hexahydronimbin is hydrogenated in acetic acid in presence of platinum oxide. That the multiplet (2H, τ 6·8) arising from the two C-11 hydrogens in the diketone XV (*v. infra*) collapses to a broadened singlet on irradiation of H-9 (τ 7·62) provides additional support.

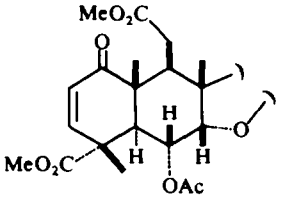
An unforseen but very revealing discovery now enabled us to relate the secondary acetate and one terminus of the cyclic ether of nimbin to the part structure VIII. An attempt to acetylate the gummy hydroxy-dicarboxylic acid (XVI), obtained by hydrolysis of hexahydronimbin (XII) afforded the dilactone (XVII), $C_{26}H_{32}O_6$, m.p. 252–254°, $[\alpha]_D$ +13°, $\nu_{max}$ ($CCl_4$) 1797 (enol-δ-lactone) and 1768 (γ-lactone) $cm^{-1}$. Formation of a γ-lactone between the carboxyl which does not survive in pyronimbic acid and the secondary OH which is acetylated in nimbin, clearly places the latter at C-6 as in XVII. Attachment of ethereal oxygen at C-7 would then account for the observed AMX system [τ 5·44 (*q*)), H-6, 5·68 (*d*), H-7 and 7·18 (*d*), H-5;



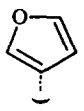

XIV



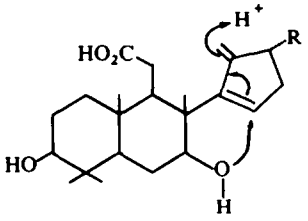

XVII




A




B



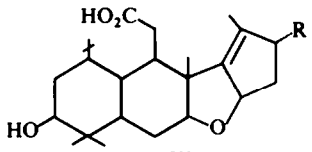

XVIII

$J_{AX}$ = 12 Hz, $J_{AM}$ = 3 Hz], which defines the configurations at H-5, H-6 and H-7 as shown, and in addition suggests the absence of hydrogens at C-8 and C-10. This AMX system is clearly present in nimbin itself (Fig. 1) and is replaced in the diketone (XV), $C_{28}H_{32}O_8$, m.p. 155–157°, $[\alpha]_D$ +139° (obtained by oxidation of desacetyl nimbin) by two singlets (1H each) at $\tau$ 5·68 (H-7) and $\tau$ 6·24 (H-5).

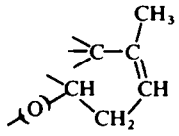
The X proton (H-5) appears at remarkably low field in nimbin ($\tau$ 6·25) and the diethyl ester ($\tau$ 6·3), but moves upfield in the decarboxylated derivative Xa ($\tau$ 7·75) and in pyronimbic acid XI ($\tau$ 7·15).

At this point we had firmly established the part-structures (A) and (B), accounting for the moiety $C_{24}H_{29}O_9$ of nimbin. The remaining undefined fragment, $C_6H_7$, is known from the NMR spectrum to contain a methyl ethylene and the other (secondary) terminus of the oxide ring. Both can be rationally derived if it is assumed that (a) C-12/C-13 fission in an aopeuphol (XIII) precursor leads to the diene-carboxylic acid (XVIII) and (b) the C-7 OH adds terminally to the conjugated diene at C-15, leading to XIX and by secondary modifications (some, or all of these may, in fact, precede formation of XIX) to nimbin (I).

However, we were debarred from accepting this obvious and simple hypothesis by a misinterpretation of the NMR spectrum of nimbin which, at the time of our preliminary communication,[1] appeared to point firmly to the rationally irreconcilable part-structure (XX) for the missing fragment.




XIX




XX

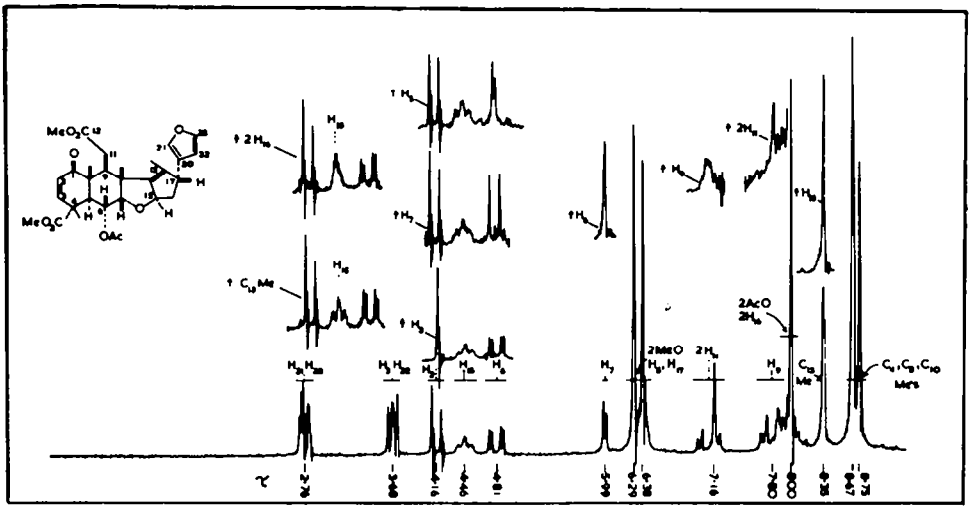


FIG. 1. NMR Single and Double Resonance Spectra at 100 MHz of Nimbin (I).

The correct interpretation of the NMR spectrum and the consequently acceptable structure (I) for nimbin were advanced[7] very soon after our announcement by Narayanan, *et al.*

We were soon convinced that their interpretation was sound, when we examined in much greater detail than had been possible with nimbin, the NMR spectrum of salannin (see Part IX[8]), which contains the same structural element that had misled us in nimbin. The relevant discussion is deferred to the following paper.[8]

We prefer[9] the α-configuration for the methoxycarbonyl group at C-4 because of the ease of γ-lactone formation in the dilactone (XVII), and because of the situation in salannin for which there is additonal support. The configurations at C-15 and C-17 are based on the NMR analysis of salannin.[8] Narayanan and his colleagues have recently submitted evidence which leads them to assign the same configurations at C-4[10] and C-15, C-17.[11] The ORD curve of pyronimbic acid has been interpreted[12] as favouring the absolute configuration of nimbin depicted in I.

## EXPERIMENTAL

For general experimental see Part I.[13] NMR spectra were obtained on a Varian 4311 NMR spectrometer operating at 56·4 Hz, and adapted for double resonance using the single side-band technique developed[14] by Turner.

*Isolation of nimbin and salannin.* Nim-oil (1 kg) (obtained through the good offices of Dr. T. F. Macrae, Glaxo Laboratories, Greenford) was dissolved in benzene–light petroleum (1:3; 1 l.) and chromatographed over alumina (3 kg Spence H deactivated with 240 ml of 10% acetic acid in water). The column was washed with light petroleum (4 l.) which eluted fats and then ether–light petroleum (1:9 to 9:1) to afford nimbin (540 mg). The residue (170 g) obtained by removal of solvents from the fractions that did not crystallize spontaneously, was rechromatographed on Spence alumina (3 kg) deactivated as above. The column was eluted with light petroleum, ether–light petroleum (1:9) and finally by gradient elution [ether (3 l.) into ether–light petroleum (1:5; 3 l.)]. Crystallization of the earlier fractions from MeOH afforded nimbin (1·9 gm) m.p. 201–204° (reported 205°[2]) $\nu_{max}$ ($CCl_4$) 1739 (carbomethoxyls and acetate) 1688 (α,β-unsaturated ketone) $cm^{-1}$.

Crystallization of the later fractions from EtOAc–light petroleum afforded *salannin* (9·45 gm) m.p. 167–170°, $[\alpha]_D$ +167° (*c*, 1·2 in $CHCl_3$), $\nu_{max}$ ($CCl_4$) 1710 (tiglate), 1743 (acetate and carbomethoxyl) $cm^{-1}$. (Found: C, 68·5; H, 7·5. $C_{34}H_{44}O_9$ requires: C, 68·45; H, 7·45%.)

*Diethyl nimbate acetate* (IX). Nimbic acid[3] (138 mg) was dissolved in EtOH, an excess of ethereal diazoethane added and the soln kept at 20° for 12 hr. Removal of solvent gave a gum (145 mg) which was acetylated with $Ac_2O$ (2 ml) and pyridine (2 ml) for 3 hr at 95°. The product, obtained as usual, was chromatographed on Grade IV acid alumina. Benzene eluted material (120 mg) which crystallized from EtOAc–light petroleum to afford *diethyl nimbate acetate* (IX) (70 mg), m.p. 174–176°, $[\alpha]_D$ +106° (*c*, 1·2). (Found: C, 67·8; H, 7·35. $C_{32}H_{40}O_9$ requires:: C, 67·6; H, 7·1%.)

*Desacetyl nimbin.*[3,4] Nimbic acid[3] (100 mg) was dissolved in MeOH and an excess of ethereal diazomethane added. Removal of solvent afforded desacetyl nimbin (80 mg), m.p. 211–214° (lit 215–216°) (from EtOAc–light petroleum), $\nu_{max}$ ($CCl_4$) 1687 (α,β-unsaturated ketone), 1743 (carbomethoxyls) $cm^{-1}$.

*Oxidation of desacetyl nimbin*

*The diketone* (XV). To desacetyl nimbin (80 mg) in pyridine (2 ml) was added a soln of $CrO_3$ (200 mg) in pyridine (4 ml). After 2 hr at 20° MeOH (0·5 ml) was added and the mixture worked-up in the usual way to afford a yellow oil which was chromatographed over Grade IV acid alumina (3·5 g). Benzene eluted, first, a colourless "jelly" (20 mg) which was not further investigated and then the crystalline *diketone* (XV; 35 mg), m.p. 154–157° (from EtOAc–light petroleum), $[\alpha]_D$ +139° (*c*, 1·4) $\nu_{max}$ (Nujol) 1680 (α,β-unsaturated ketone), 1730 (cyclohexanone, carbomethoxyls) $cm^{-1}$. (Found: C, 67·70; H, 6·80. $C_{28}H_{32}O_8$ requires: C, 67·75; H, 6·50%.)

*Decarboxylation of nimbin.*[4] Nimbin (1 g) in 5% methanolic KOH aq (50 ml) was refluxed in a $N_2$ atm for 1 hr. The usual work-up afforded a yellow gum (910 mg) which was dissolved in MeOH (10 ml) and methylated with an excess of diazomethane. The mixture of esters was chromatographed on Grade III acid alumina. The column was eluted with light petroleum–benzene (1:1) and then by gradient elution [ether (2 l.) into ether–light petroleum (1:19, 1·5 l.)], 50 ml fractions being taken. The fractions 27–29 contained ester A[4] (57 mg), m.p. 138–142° (from ether–light petroleum) (lit 138–139°), $\nu_{max}$ (Nujol) 3500 (hydroxyl), 1735 (carbomethoxyl), 1710 (cyclohexanone) $cm^{-1}$. The fractions 33–35 contained ester B[4] (72 mg), m.p. 153–155° (from ether–light petroleum) (lit 153–154°), $\nu_{max}$ (Nujol) 3500 (OH), 1740 (carbomethoxyl), 1690 (α,β-unsaturated ketone) $cm^{-1}$. Crystallization of fraction 36 from ether–light petroleum afforded ester C[4] m.p. 153–156°, (lit 158–160°), $\nu_{max}$ (Nujol) 3500 (OH), 1740 (carbomethoxyl), 1690 (α,β-unsaturated ketone) $cm^{-1}$.

*Acetate of ester B* (Xa). Ester B (72 mg) was dissolved in pyridine (1 ml) and $Ac_2O$ (1 ml), and the soln heated in a $N_2$ atm at 95° for 1·5 hr. Removal of solvent afforded a gum (65 mg) which was chromatographed on Grade IV acid alumina. Benzene eluted *the acetate of ester B* (Xa; 57 mg), m.p. (from chloroform–light petroleum) 178–180° (with previous sublimation at 165–168°), $[\alpha]_D$ +178° (*c*, 2·0). (Found: C, 69·75; H, 7·25. $C_{28}H_{34}O_7$ requires: C, 69·7; H, 7·1%.)

*Hexahydronimbin.* A soln of nimbin (520 mg) in EtOAc (20 ml) was shaken in an atm of $H_2$ in the presence of 10% Pd–C (315 mg) for 12 hr at atm press. The uptake of $H_2$ was 69 ml. The soln was filtered and the solvent removed *in vacuo* to afford hexahydronimbin (510 mg) which, crystallized from EtOAc–light petroleum, had m.p. 202–206° $\lambda_{max}$ 213 nm (log ε 3·69). (Narasimhan[3] quotes for "tetrahydronimbin" m.p. 207°.)

*Hydrogenation of hexahydronimbin*

*The δ-lactone* (XIV). Hexahydronimbin (155 mg) in AcOH (20 ml) was hydrogenated in presence of Adams' catalyst (360 mg) at atm press for 16 hr at 20°. The soln was filtered and the solvent removed *in vacuo* to give the saturated *δ-lactone* (XIV), m.p. 269–271° (from EtOAc–ether), $\nu_{max}$ ($CCl_4$) 1755 (δ-lactone), 1738 (carbomethoxyl and acetate) $cm^{-1}$. (Found: C, 66·8; H, 8·35. $C_{29}H_{42}O_8$ requires: C, 67·15; H, 8·15%.)

*The dilactone* (XVII). Hexahydronimbin (200 mg) was hydrolysed with 10% methanolic KOH at 20° for 5 hr. The soln was acidified with 6N HCl and extracted with EtOAc. Removal of solvent afforded a gum (195 mg) which was kept with $Ac_2O$ (3 ml) and pyridine (4·5 ml) for 2 hr at 110°. The gummy product was chromatographed on Grade IV acid alumina. Benzene–$CHCl_3$ (1:1) eluted a yellow gum (130 mg) which crystallized from EtOAc to give the *dilactone* (XVII; 105 mg) m.p. 252–254°, $[\alpha]_D$ +13° (*c*, 1·4), $\nu_{max}$ ($CCl_4$) 1797 (enol-δ-lactone), 1768 (γ-lactone) $cm^{-1}$. (Found: C, 70·65; H, 7·75. $C_{26}H_{32}O_6$ requires: C, 70·9; H, 7·3%.)

*Acknowledgement*—One of us (M.H.) thanks the S.R.C. for a maintenance award.

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