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TERPENOIDS AND RELATED COMPOUNDS—II[1]

INVESTIGATIONS ON THE STRUCTURE OF NIMBIN[2]

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Abstract—Nimbin, the crystalline principle of *M. azadirachta* Linn. is assigned the molecular formula $C_{29}H_{36}O_8$. 1,2,5-Trimethylnaphthalene was isolated from the dehydrogenation of a reduction product of nimbin. The products of alkaline degradation and pyrolytic experiments establish that nimbin possesses either of the partial structures X or XI.

IN a previous communication[1] we reported investigations on the constituents of the trunk bark of *Melia azadirachta* Linn. and the structure of nimbiol, the new keto-phenol isolated. The present communication deals with investigations on nimbin, the bitter principle of the tree, isolated by Siddiqui *et al.* from the seed-oil,[3] the blossoms,[4] the root bark[5] and the trunk bark[6] and given the molecular formula, $C_{28}H_{40}O_8$. Later Mitra[7] suggested the molecular formula, $C_{28}H_{38}O_8$ ($\pm CH_2$) for nimbin, and isolated acetic acid, methanol, nimbinic acid, m.p. 263°, Neutral A or desacetyl nimbin, m.p. 215° and nimbic acid, m.p. 160–162° from the alkaline hydrolysis. Nimbinic acid on esterification gives Neutral A, which on acetylation gives nimbin. Mitra[7] suggested the presence of (a) an aldehyde, (b) an acetoxy, (c) a carbomethoxy, (d) an $\alpha\beta$-unsaturated lactone and (e) an isolated methoxy group in nimbin. Narasimhan[8] on the other hand suggested the molecular formula $C_{30}H_{36}O_9$ and agreed that nimbin has an acetoxy, a carbomethoxy and an isolated methoxy group and further suggested that nimbin has an $\alpha\beta$-unsaturated ketone, another $\alpha\beta$-unsaturated carbomethoxy group as well as a furan ring. On hydrogenation nimbin gives dihydronimbin and tetrahydronimbin. This worker further established that in dihydronimbin the ethylenic linkage in conjugation with the ketonic carbonyl group has been hydrogenated. An investigation on the nimbic acid of Mitra led Narasimhan to suggest that this acid is dibasic, and on sublimation loses water and carbon dioxide to give an enol-lactone, 'pyronimbic acid'. Later Mitra[9] provisionally suggested a 1-carbomethoxy steroid type structure for nimbin without apparent reason.

In continuation of the work on *M. azadirachta* Linn.[1] we selected the trunk bark as the source of nimbin. The neutral fraction of the benzene extract of the trunk bark was subjected to partition between petroleum ether and 80 per cent aqueous methanol.[1] The methanolic extract on acetylation and chromatography yielded nimbin, m.p. 204-205°, $[\alpha]_D$ +168°, in ca. 0·08% yield. The bitterness of nimbin is very mild, whereas that of its various derivatives is quite strong.

[1] Part I: P. Sengupta, S. N. Choudhuri and H. N. Khastgir, *Tetrahedron* **10**, 45 (1960).
[2] Preliminary investigations on nimbin were published in short communications: [a] P. Sengupta, S. K. Sengupta and H. N. Khastgir, *Chem & Ind.* 1402 (1958); [b] 397 (1959) and [c] 1194 (1959).
[3] S. Siddiqui, *Curr. Sci.* **11**, 278 (1942).
[4] C. Mitra, P. N. Rao, S. Bhattacharjee and S. Siddiqui, *J. Sci. & Ind. Res. India* **6B**, 19 (1947).
[5] C. Mitra, P. N. Rao and S. Siddiqui, *J. Sci. & Ind. Res. India* **12B**, 152 (1953).
[6] S. Bhattacharjee, C. Mitra and S. Siddiqui, *J. Sci. & Ind. Res. India* **12B**, 154 (1953).
[7] C. Mitra, *J. Sci. & Ind. Res. India* **15B**, 425 (1956).
[8] N. S. Narasimhan, *Chem. & Ind.* 661 (1957).
[9] C. Mitra, *J. Sci. & Ind. Res. India* **16B**, 477 (1957).

The analytical data of nimbin and its degradation products suggest the molecular formula, $C_{29}H_{36}O_9$ and indicate the presence of two methoxy groups in the molecule, in agreement with the observation of Mitra[7], but in contrast to the observation of Narasimhan[8]. The ultra-violet absorption spectra of nimbin show two maxima, one at 210 mμ, which may be a composite one of isolated chromophores, an $\alpha\beta$-unsaturated ketone, a furan and one or two ethylenic linkages, while the second one at 330 mμ is due to the $\alpha\beta$-unsaturated ketone.[8] In the infra-red spectra nimbin shows peaks at 1687 ($\alpha\beta$-unsaturated ketone), 1730 (esters and acetate) and 1510 and 875 cm^{-1} (β-substituted furan[10,11]). The ultra-violet absorption spectra of dihydronimbin,[8] $C_{29}H_{38}O_9$ show two maxima, at 210 mμ and 298 mμ and the infra-red spectra show peaks at 1740 (carbonyl) and 1500 and 872 cm^{-1} (β-substituted furan).

Both nimbin and dihydronimbin show infra-red peaks around 1245 cm^{-1} indicating the presence of acetoxy groups. These groups could not be determined due to decomposition and frothing, but on hydrolysis the acetic acid generated was identified as *p*-bromophenacyl ester.

On heating under reflux with methanolic potassium hydrogen carbonate, nimbin gives nimbinic acid,[7] $C_{26}H_{32}O_8$, m.p. 259–261°d, which shows infra-red peaks at 3420 (hydroxyl), 1743, 1718 and 1677 (carbonyl) and 1505 and 875 cm^{-1} (furan). On titration it is found to be monocarboxylic and on esterification it gives methyl nimbinate, $C_{27}H_{34}O_8$, m.p. 215–216°c which on acetylation gives nimbin. Thus nimbinic acid is formed from nimbin by the hydrolysis of an acetoxy and a carbomethoxy group. The infra-red spectra of methyl nimbinate show peaks at 3420 (hydroxyl), 1730, 1715 and 1680 (carbonyl) and 1505 and 875 cm^{-1} (furan).

In addition to these infra-red peaks, nimbinic acid and methyl nimbinate show peaks at 1225 and 1230 cm^{-1} respectively, which led to the erroneous conclusion[2b] that nimbin has yet another acetoxy group. But as work progressed, we agreed[2c] with Narasimhan that nimbin has one acetoxy group and two carbomethoxy groups.

Nimbic acid prepared according to the method of Narasimhan melted at 185°d instead of 160–161°d as reported by him. A sample of nimbic acid supplied by Dr. Narasimhan after drying melted at 185°d. On esterification this acid gives methyl nimbate or desacetyl nimbin, $C_{27}H_{34}O_8$[2c] which is identical with our methyl nimbinate. Thus we agree with Narasimhan regarding the functions of eight out of the nine oxygen atoms of nimbin. These are a β-substituted furan, an acetoxy, two carbomethoxy and an $\alpha\beta$-unsaturated ketone groups. We do not agree with the view[8] that the ninth oxygen atom is present in an isolated methoxy group. Nimbin shows the presence of two methoxy groups that are accounted for by the presence of two carbomethoxy groups in the molecule.

Nimbin on reduction with lithium aluminium hydride yields a gummy product (λ_{max} 206 mμ), which did not crystallize even after acetylation and chromatography. The gummy product on dehydrogenation[2b,c] with selenium, gives 1,2,5-trimethylnaphthalene identified by its ultra-violet absorption spectra and by melting point and mixed melting point of its 1,3,5-trinitrobenzene adduct with that of an authentic specimen. Independently, however, Narasimhan[12] isolated presumably the same

[10] T. Kubota, *Tetrahedron* **4**, 68 (1958).
[11] F. M. Dean and T. A. Geissman, *J. Org. Chem.* **23**, 596 (1958).
[12] N. S. Narasimhan, *Ber. Dtsch. Chem. Ges.* **92**, 769 (1959).

naphthalene derivative, which he suggested, is 1,2,5- or 1,4,5-trimethylnaphthalene. The isolation of 1,2,5-trimethylnaphthalene indicates that nimbin is probably dicarbocyclic.

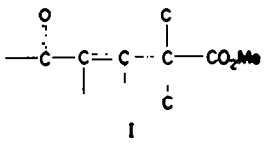
Nimbin on standing with cold methanolic potassium hydroxide gives only nimbic acid,[8] but on heating under reflux with methanolic alkali, it gives a mixture of acids from which no single component could be crystallized. The acid mixture was esterified and after chromatography over alumina yielded three crystalline esters,[2] (i) Ester A, m.p. 138–139°, $[\alpha]_D$ + 130°,(ii) Ester B, m.p. 153–154°, $[\alpha]_D$ + 140° and (iii) Ester C, m.p. 158–160°, $[\alpha]_D$ – 302°. The Esters A, B and C show molecular weights (Rast) of 382, 363 and 372 respectively, and from analyses are found to be isomeric and were first assigned[2b] the molecular formula, $C_{22}H_{28}O_5$ (or $C_{21}H_{26}O_5$). This molecular formula necessitated the assumption that during alkaline degradation, a C_4 (or C_5) unit had split off from nimbin in addition to the hydrolysis of the two carbomethoxy and the acetoxy groups. Repeated attempts to isolate this small unit proved abortive, and it was found necessary to recheck the molecular formula of these esters by other methods some of which were unsuccessful. Ester B on treatment with methanolic potassium hydrogen carbonate, yields Acid B, m.p. 213–214°, with equivalent weight (420) and molecular formula $C_{24}H_{30}O_6$. On esterification this acid gives Ester B, with molecular formula, $C_{25}H_{32}O_6$. Hence the isomeric (see below) Ester A and Ester C have the same formula.

The difference between nimbic acid and Acid B is a molecule of carbon dioxide. It is evident then that during alkaline degradation, nimbin first forms nimbic acid which is decarboxylated by the action of hot alkali to give Acid B and its isomers. Nimbic acid on treatment with hot methanolic alkali gives a mixture of acids which on esterification and chromatography yields Esters B and C. Ester C on treatment with methanolic potassium hydrogen carbonate gives an amorphous acid m.p. 180–203°, which cannot be crystallized but which on esterification yields Ester C.

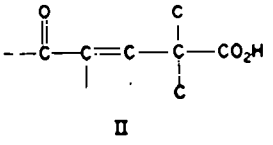
In the infra-red spectra Ester A shows peaks at 3510 (hydroxyl), 1735 (saturated ester), 1715 (saturated ketone) and 1505 and 875 cm^{-1} (furan). This fact coupled with the ultra-violet absorption spectra (maxima at 207 mμ and 297 mμ) indicates that the ketonic carbonyl group is no longer conjugated in Ester A. Ester B shows infra-red peaks at 3600 (hydroxyl), 1740 (saturated ester), 1690 ($\alpha\beta$-unsaturated ketone) and 1505 and 875 cm^{-1} (furan) and ultra-violet absorption maxima at 212 mμ and 327 mμ. Finally, Ester C shows infra-red peaks at 3520 (hydroxyl), 1735 (saturated ester), 1678 ($\alpha\beta$-unsaturated ketone) and 1502 and 875 cm^{-1} (furan) and ultra-violet absorption maxima at 214 mμ and 327 mμ.

In order to settle the interrelation of these three esters, each was treated with hot methanolic potassium hydroxide. In each case after esterification and chromatography, almost equal amounts of Esters B and C were isolated. Thus Esters B and C have an $\alpha\beta$-unsaturated ketonic system and are epimeric at the carbon atom γ to the ketone, from where the carboxylic group of nimbic acid is split off. Ester A in which the ethylenic linkage is no longer conjugated to the ketone, is evidently the $\beta\gamma$-unsaturated isomer of Esters B and C. These reactions can be explained if nimbin has the partial structure I, which on hydrolysis first gives nimbic acid (II). The latter is decarboxylated yielding as shown Ester A (III) and Esters B and C (IV, epimeric at the carbon atom marked with asterisk).

Ester B on catalytic hydrogenation yields dihydro-Ester B, $C_{25}H_{34}O_6$, m.p.



I



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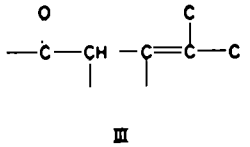
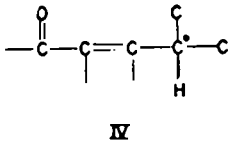
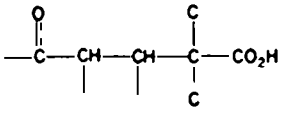


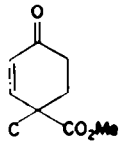
III



IV



V



VI

137–139°, $[\alpha]_D$ +176° having infra-red peaks at 3580(hydroxyl), 1728(saturated ester), 1702 (saturated ketone) and 1500 and 878 cm $^{-1}$ (furan). Similarly Ester C on hydrogenation gives dihydro-Ester C, $C_{25}H_{34}O_8$, m.p. 150–151°, $[\alpha]_D$ −197° having infra-red peaks at 3550 (hydroxyl), 1728 (saturated ester), 1700 (saturated ketone) and 1500 and 874 cm $^{-1}$ (furan). These two dihydroesters in which the ethylenic linkage conjugated to the ketonic carbonyl has been hydrogenated, can not be epimerized. This fact proves that epimerization only takes place at the carbon atom marked with asterisk in the partial structure IV.

Dihydronimbin on similar treatment with methanolic alkali gives only one dihydroacid, $C_{25}H_{32}O_8$, m.p. 192–195°d, $[\alpha]_D$ +229° in which both the carboxylic groups are retained. This dihydroacid has the partial structure V. On esterification this acid gives desacetyl dihydronimbin, $C_{27}H_{36}O_8$, m.p. 146–147° which on acetylation gives dihydronimbin.

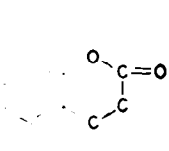
The infra-red spectra of dihydro-Esters B and C (peaks around 1700 cm^{-1}) suggest that the ketonic group of nimbin is in a six membered ring. The partial structure of nimbin can now be enlarged to structure VI. This partial structure, however, needs clarification on an important point. Desacetyl nimbin has two ester peaks at 1730 and 1715 cm^{-1} in the infra-red spectra. The former peak is undoubtedly due to a saturated carbomethoxy group, but the latter peak, which disappears after decarboxylation, has been attributed to an $\alpha\beta$-unsaturated carbomethoxy group by Narasimhan.[8] This fact cannot explain the facile decarboxylation with methanolic base. The latter peak (1715 cm $^{-1}$) is therefore also due to a saturated carbomethoxy group, which is vinylogously situated with respect to the $\alpha\beta$-unsaturated ketonic carbonyl system. Since on decarboxylation an epimeric centre is formed, the carbon atom α to the carbomethoxy group in nimbin does not contain any hydrogen atom (Structure VI). It was shown by Cole and Thornton[13] that in triterpenoids the saturated carbomethoxy peak in the infra-red appears in the region 1714 to 1725 cm^{-1}.

[13] A. R. H. Cole and D. W. Thornton, *J. Chem. Soc.* 1007 (1956).

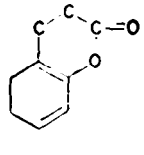
That Esters A, B and C still retain the original carbocyclic ring system is proved by the dehydrogenation of the lithium aluminium hydride reduction product of a mixture of these three esters. The naphthalene derivative isolated from the dehydrogenation was identified as 1,2,5-trimethylnaphthalene.

The reactions of the hydroxyl group in Ester B proved to be secondary in nature. Ester B on acetylation gives a monoacetate, $C_{27}H_{34}O_7$, m.p. 174–175°. On chromium trioxide oxidation by Sarett's method,[14] Ester B gives a diketoester, $C_{25}H_{30}O_6$, m.p. 177°, which does not have any hydroxyl peak in the infra-red, but has peaks at 1710 (saturated ketone and ester), 1680 ($\alpha\beta$-unsaturated ketone) and 1500 and 875 cm^{-1} (furan). The ultra-violet absorption spectra of this diketoester show maxima at 206 and 309 mμ. Desacetyl dihydronimbin also on similar oxidation with chromium trioxide[14] gives a diketodiester, $C_{27}H_{34}O_8$, m.p. 155–156°.

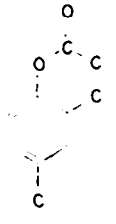
Narasimhan suggested that 'pyronimbic acid', which is formed from nimbic acid and whose infra-red spectra indicate the absence of the ketonic peak at 1687 cm^{-1} has the structure VII or VIII. On repeating the work of Narasimhan, the same enol-lactone was isolated and assigned the molecular formula, $C_{24}H_{28}O_5$. This enol-lactone shows maxima in the ultra-violet at 207 and 278 mμ. It dissolves in methanolic



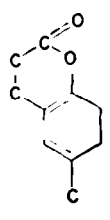
VII



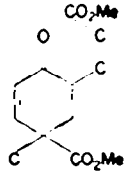
VIII



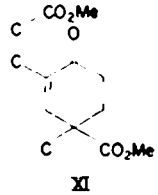
IX a



IX b



X



XI

potassium hydroxide solution and on acidification yields a mixture of acids, which on esterification followed by chromatography furnish Esters B and C. Individually Acids B and C were subjected to sublimation in vacuum and in each case 'pyronimbic acid' was isolated and identified. It is thus evident that in 'pyronimbic acid' the asymmetry of the epimeric centre marked with asterisk in the partial structure IV has been destroyed. Since the ketone takes part in the enol-lactone formation, the partial

[14] G. I. Poos, W. F. Johns and L. H. Sarett, *J. Amer. Chem. Soc.* 77, 1026 (1955).

structure of 'pyronimbic acid' is either IXa or IXb and not of the types suggested by Narasimhan. These experiments also establish that nimbin has either the partial structure X or XI.

EXPERIMENTAL

The pet ether b.p. 60–80° was used throughout the investigation. The ultra-violet absorption spectra of all the compounds were studied in 95% ethyl alcohol.

Isolation of nimbin

Dried and powdered trunk bark of *M. azadirachta* Linn. (3·6 Kg) was extracted in a Soxhlet with benzene for 20 hr. The residue from the benzene extract was taken up in ether, washed (5% NaOH, and water) and dried (Na_2SO_4). The gummy material (35 g) was partitioned between pet ether and 80% aqueous methanol. The residue (16·8 g) from the methanol layer was acetylated with pyridine (30 cc) and acetic anhydride (30 cc). The crude product (16 g) was chromatographed over alumina (200 g, deactivated with 8 cc of 10% aqueous acetic acid). The crystalline material (4·6 g) eluted with a mixture of pet ether and benzene (1:1) on crystallization from methanol afforded nimbin (2·8 g), m.p. 204–205°, $[\alpha]_D$ −168° ($CHCl_3$). (Reported[7] m.p. 205°, $[\alpha]_D$ −170°.) (Found: C, 65·95; H, 6·79. OCH_3, 11·75. $C.CH_3$, 9·33. $C_{28}H_{34}O_8$ requires: C, 65·98; H, 6·87. OCH_3 (two), 11·7. $C\text{-}CH_3$ (two), 10·19%).

Mol. wt., Found (Rast), 530. Calc., 528.

U.V. λ_{max} 210 mμ (E 32,700) and 330 mμ (E 66).

I.R. ($CHCl_3$) Peaks at 1730 (ester), 1687 (α,β-unsaturated ketone) and 1510 and 875 cm^{-1} (β-substituted furan).

(Nujol) Peaks at 1732 (ester), 1700 (α,β-unsaturated ketone), 1246 (acetate) and 1510 and 875 cm^{-1} (β-substituted furan).

Dihydronimbin

A solution of nimbin (0.5 g) in ethyl acetate (20 cc) was stirred in an atmosphere of hydrogen in presence of 10% palladium charcoal catalyst (0·1 g) until one mole equivalent of hydrogen was absorbed. The solid material (0·48 g), m.p. 208–210° isolated on crystallization from methanol gave dihydronimbin (0·4 g), m.p. 215–216° (reported[8] m.p. 218°), $[\alpha]_D$ −167·5° ($CHCl_3$). (Found: C, 65·70; H, 7·30. $C_{28}H_{36}O_8$ requires: C, 65·64; H, 7·22%)

U.V. λ_{max} 210 mμ (E 16,200) and 298 mμ (E 29)

I.R. ($CHCl_3$) Peaks at 1740 (carbonyl), 1245 (acetate) and 1500 and 872 cm^{-1} (β-substituted furan).

Isolation of acetic acid (as p-bromophenacyl ester) from the alkaline hydrolysis of nimbin

A solution of nimbin (4·67 g) in 5% methanolic potassium hydroxide (200 cc) was refluxed for 2 hr, and acidified with dil HCl and the precipitated solid filtered. The filtrate was adjusted to pH 8, concentrated to a small volume, filtered, acidified with dil H_2SO_4 and extracted with ether. The residual gummy acid mixture (1·3 g) was dissolved in ethyl alcohol (10 cc) and adjusted to pH 7·5 with dil NaOH. The solution was refluxed for 2 hr after the addition of a solution of *p*-bromophenacyl bromide (1·5 g) in ethyl alcohol (20 cc). A gummy residue was chromatographed over alumina (150 g, deactivated with 6 cc of 10% aqueous acetic acid). A mixture of pet ether and benzene (4:1) eluted a solid (0·44 g), m.p. 76–82°, which on crystallization from ethyl alcohol melted at 83–84°. Its mixed m.p. with an authentic specimen of *p*-bromophenacyl ester of acetic acid showed no depression.

Hydrolysis of nimbin with methanolic potassium hydrogen carbonate

Nimbinic acid. A mixture of nimbin (1 g), methanol (20 cc), potassium hydrogen carbonate (1·33 g) and water (6·6 cc) was refluxed for 1½ hr, diluted with water and extracted with ether. The alkaline aqueous layer was acidified with cold dil HCl and the precipitated solid extracted with ether, washed with water and dried (Na_2SO_4). On concentration to a small volume, a solid (0·24 g), m.p. 245–250° crystallized out, and recrystallization from ether gave nimbinic acid, m.p. 259–261°d, $[\alpha]_D$ −112° ($CHCl_3$). (Reported[7] m.p. 263°, $[\alpha]_D$ −129·5°). (Found: C, 65·58; H, 6·85. $C_{28}H_{32}O_8$ requires: C, 66·08; H, 6·83%). Mol. wt., Found (titration at 0°), 456, Calc., 472.

U.V. λ_{max} 208 mμ (E 24,400) and 330 mμ (E 70).

I.R. ($CHCl_3$) Peaks at 3420 (hydroxyl), 1743, 1718 and 1677 (carbonyl) and 1505 and 875 cm^{-1} (β-substituted furan).

Esterification of nimbinic acid

Desacetylnimbin. Nimbinic acid (100 mg) in a mixture of ether and methanol was esterified with an ethereal solution of diazomethane (from 100 mg of nitrosomethyl urea). The ester (90 mg), m.p. 207 210° on crystallization from methanol gave *desacetylnimbin*, m.p. 215–216° (reported[7] m.p. 210 214°), $[\alpha]_D$ +105° ($CHCl_3$). (Found: C, 67·10; H, 6·90. $C_{27}H_{34}O_8$ requires: C, 66·65; H, 7·04%).

I.R. ($CHCl_3$) Peaks at 3420 (hydroxyl), 1730, 1715 (ester), 1680 ($\alpha\beta$-unsaturated ketone) and 1505 and 875 cm^{-1} (β-substituted furan).

Acetylation of desacetylnimbin

Nimbin. Desacetylnimbin (130 mg) was acetylated with pyridine (1·3 cc) and acetic anhydride (1·3 cc) and the crude product (120 mg) on crystallization from methanol gave *nimbin*, m.p. and mixed m.p. 204–205°.

Hydrolysis of nimbin with cold methanolic potassium hydroxide

Nimbic acid. Nimbic acid was prepared from nimbin (2 g) by treatment with cold methanolic potassium hydroxide solution[12]. The crude acid, m.p. 150 151°d, on crystallization from aqueous methanol melted at 159 160°, but after drying melted at 184 185°d, $[\alpha]_D$ +157° (pyridine). (Found: C, 65·12; H, 6·78. $C_{28}H_{30}O_8$ requires: C, 65·49; H, 6·60%).

U.V. λ_{max} 209 mμ (E 14,300) and 327 mμ (E 50).

I.R. (Nujol) Peaks at 3500 (hydroxyl), 1718 (carboxyl), 1680 ($\alpha\beta$-unsaturated ketone) and 1500 and 872 cm^{-1} (β-substituted furan).

Esterification of nimbic acid

Desacetylnimbin. Nimbic acid on esterification gave methyl nimbate, m.p. 212·214°, which was identical with desacetylnimbin (mixed m.p. and infra-red) prepared from nimbinic acid.

Lithium aluminium hydride reduction of nimbin and dehydrogenation of the product

To a cold suspension of lithium aluminium hydride (1·2 g) in dry ether (150 cc) was added a solution of nimbin (1 g) in tetrahydrofuran (20 cc) and dry ether (50 cc). The reaction mixture was kept in the ice bath for 20 min, then refluxed for 5 hr, and treated with water. The precipitated aluminium oxide was filtered and washed with hot chloroform. The combined filtrates were dried (Na_2SO_4) and evaporated to a gummy residue (1·6 g, λ_{max} 206 mμ), which did not crystallize even after acetylation and chromatography.

The gummy reduction product (1 g) was heated with selenium powder (3 g) at 320 340° for 20 hr, and the product ether extracted. The ether solution was washed (10% NaOH and water), dried (Na_2SO_4) and evaporated to give a gummy residue (0·2 g), which was chromatographed over alumina (10 g). The colourless oil (0·07 g) eluted with pet ether was dissolved in methanol and treated with a solution of 1,3,5-trinitrobenzene (0·07 g) in methanol. The product (0·02 g), m.p. 145–148°, on recrystallization from methanol gave orange-red crystals, m.p. 152 154° identical by mixed m.p. with the adduct of 1,3,5-trinitrobenzene and 1,2,5-trimethylnaphthalene. (Found: C, 59·60; H, 4·72. Calc. for $C_{19}H_{17}O_6N_3$: C, 59·53; H, 4·47%).

1,2,5-Trimethylnaphthalene regenerated from the above adduct by passage through a column of alumina with pet ether, had the ultra-violet absorption maxima at 230 mμ (log E 4·86), 278 mμ (log E 3·69), 288 mμ (log E 3·80), 324 mμ (log E 2·85) and 355 mμ (log E 1·22).

Preparation of esters A, B *and* C

Nimbin (2 g) in 5% methanolic potassium hydroxide solution (100 cc) was refluxed for 1 hr, poured into water and extracted with ether. The alkaline aqueous solution was acidified with cold dil HCl, with separation of yellowish white solids. The crude mixture melted over a range (90–140°) and could not be purified by crystallization.

A suspension of the crude mixture of acids in ether was treated with an ethereal solution of

diazomethane (from 1 g of nitrosomethylurea) and kept overnight at room temp. The gummy mixture of esters (1·43 g) was chromatographed over alumina (100 g, deactivated with 4 cc of 10% aqueous acetic acid) and esters A, B and C isolated as follows.

Ester A. The crystalline material eluted with pet ether and benzene (3:2), on crystallization from pet ether and ether afforded Ester A (0·07 g), m.p. 138–139°, $[\alpha]_D$ +130° ($CHCl_3$). (Found: C, 70·47; H, 7·44. $C_{28}H_{32}O_6$ requires: C, 70·07; H, 7·53%). Mol. wt., Found (Rast), 382. Calc., 428.

U.V. λ_{max} 207 mμ (E 13,800) and 297 mμ (E 87).

I.R. ($CHCl_3$) Peaks at 3510 (hydroxyl), 1735 (ester), 1714 (saturated ketone) and 1505 and 875 cm^{-1} (furan).

Ester B. The crystalline material eluted with pet ether and benzene (2:3), on crystallization from pet ether and ether afforded Ester B (0·26 g), m.p. 153–154°, $[\alpha]_D$ +140° ($CHCl_3$). (Found: C, 70·45; H, 7·43. $C_{28}H_{32}O_6$ requires: C, 70·07; H, 7·53%). Mol. wt., Found (Rast), 363. Calc., 428.

U.V. λ_{max} 212 mμ (E 14,300) and 327 mμ (E 67).

I.R. ($CHCl_3$) Peaks at 3600 (hydroxyl), 1740 (ester), 1690 (αβ-unsaturated ketone) and 1505 and 875 cm^{-1} (furan).

Ester C. The crystalline material eluted with benzene, on crystallization from pet ether and ether afforded Ester C (0·28 g), m.p. 158–160°, $[\alpha]_D$ +302° ($CHCl_3$). (Found: C, 70·55; H, 7·50. $C_{28}H_{32}O_6$ requires: C, 70·07; H, 7·53%). Mol. wt., Found (Rast), 372. Calc., 428.

U.V. λ_{max} 214 mμ (E 12,500) and 327 mμ (E 59).

I.R. ($CHCl_3$) Peaks at 3520 (hydroxyl), 1735 (ester), 1678 (αβ-unsaturated ketone) and 1502 and 875 cm^{-1} (furan).

Preparation of the monoacetate of ester B

A solution of Ester B (140 mg) in pyridine (1·4 cc) and acetic anhydride (1·4 cc) was allowed to stand overnight at room temp. The acetate m.p. 165–170° on crystallization from methanol afforded the monoacetate of Ester B (45 mg), m.p. 174–175°, $[\alpha]_D$ +205° ($CHCl_3$). (Found: C, 69·54; H, 7·30. $C_{32}H_{34}O_7$ requires: C, 68·92; H, 7·28%).

Hydrolysis of ester B *with potassium hydrogen carbonate*

Acid B. A mixture of Ester B (130 mg), potassium hydrogen carbonate (130 mg), water (1 cc) and methanol (2 cc) was refluxed for 2 hr, and extracted with ether. The aqueous alkaline solution was acidified with cold dil HCl, yielding material, m.p. 200–209° which on crystallization from dry ether gave Acid B (35 mg), m.p. 213–214°, $[\alpha]_D$ −139° ($CHCl_3$). (Found: C, 69·96; H, 7·49. $C_{24}H_{30}O_6$ requires: C, 69·54; H, 7·30%). Equiv. wt., Found (titration), 420. Calc., 414.

Esterification of acid B

Ester B. Acid B (200 mg) was esterified with an ethereal solution of diazomethane (from 200 mg of nitrosomethylurea). The crude ester (190 mg), m.p. 145–148°, on crystallization from pet ether and ether afforded Ester B, m.p. and mixed m.p. 153–154°.

Hydrolysis of ester C *with potassium hydrogen carbonate*

Amorphous acid C. A mixture of Ester C (280 mg), methanol (7·5 cc), potassium hydrogen carbonate (350 mg) and water (2·5 cc) was refluxed for 2 hr, and extracted with ether. The aqueous alkaline solution on acidification with cold dil HCl afforded amorphous Acid C (190 mg), m.p. 180–203°. All attempts at the crystallization of the solid failed.

Esterification of amphorous acid C

Ester C. Acid C (190 mg) was esterified with an ethereal solution of diazomethane (from 190 mg of nitrosomethylurea) yielding Ester C (100 mg), m.p. and mixed m.p. 158–160°.

Isomerization of ester A *to esters* B *and* C

A solution of Ester A (85 mg) in 5% methanolic potassium hydroxide solution (5 cc) was refluxed for 1 hr, then acidified with cold dil HCl, yielding material, m.p. 140–165°d. This was esterified with ethereal diazomethane (from 85 mg of nitrosomethylurea) and the ester (80 mg) chromatographed over alumina (10 g, deactivated with 0·6 cc of 10% aqueous acetic acid). The solid material,

m.p. 141–145° eluted with pet ether and benzene (3:2), crystallized from pet ether and ether as Ester B (21 mg), m.p. and mixed m.p. 151–153°. The second material eluted with benzene on crystallization from pet ether and ether afforded Ester C (20 mg), m.p. and mixed m.p. 158–160°.

Equilibration of ester B

Similarly Ester B (95 mg) with 5% methanolic potassium hydroxide solution (5 cc) gave a solid acid mixture, which on esterification and chromatography afforded Ester B (31 mg), m.p. and mixed m.p. 152–154° and Ester C (29 mg), m.p. and mixed m.p. 158–159°.

Equilibration of ester C

Ester C (100 mg) with 5% methanolic potassium hydroxide solution (5 cc) gave a solid acid mixture, which on esterification and chromatography afforded Ester B (38 mg), m.p. and mixed m.p. 152–154° and Ester C (36 mg), m.p. and mixed m.p. 158–160°.

Treatment of nimbic acid with base

Isolation of esters B *and* C. A solution of nimbic acid (1 g) in 5% methanolic potassium hydroxide solution (150 cc) was refluxed for 1 hr, and acidified with dil HCl, yielding a mixture of acids (0·7 g) which on esterification and chromatography afforded Ester B (0·17 g), m.p. and mixed m.p. 153–154° and Ester C (0·16 g), m.p. and mixed m.p. 158–160°.

Dihydro-ester B

A solution of Ester B (0·53 g) in ethyl acetate (25 cc) was stirred in an atmosphere of hydrogen in presence of 10% palladium charcoal catalyst (0·2 g) until one mole equivalent of hydrogen was absorbed. The product (0·5 g) was chromatographed over alumina (20 g, deactivated with 1·2 cc of 10% aqueous acetic acid). Elution with pet ether and benzene (4:1) gave crystalline solids (0·42 g), which on crystallization from pet ether and ether gave dihydro-Ester B, m.p. 137–139°, $[\alpha]_D$ +176° ($CHCl_3$). (Found: C, 70·12; H, 7·71. $C_{28}H_{34}O_6$ requires: C, 69·74; H, 7·96%).

U.V. λ_{max} 209 mμ (E 13,600) and 292 mμ (E 38).

I.R. ($CHCl_3$) Peaks at 3580 (hydroxyl), 1728 (saturated ester), 1702 (saturated ketone) and 1500 and 878 cm^{-1} (furan).

Dihydro-ester C

Similarly Ester C (0·51 g) in ethyl acetate (30 cc) in an atmosphere of hydrogen and in presence of 10% palladium charcoal catalyst (0·3 g) yielded a product (0·62 g) which was chromatographed over alumina and afforded dihydro-Ester C, m.p. 150–151°, $[\alpha]_D$ +197° ($CHCl_3$). (Found: C, 70·13; H, 7·66. $C_{28}H_{34}O_6$ requires: C, 69·74; H, 7·96%).

U.V. λ_{max} 210 mμ (E 12,100) and 288 mμ (E 62).

I.R. ($CHCl_3$) Peaks at 3550 (hydroxyl), 1728(saturated ester), 1700 (saturated ketone) and 1500 and 874 cm^{-1} (furan).

Attempted equilibration of dihydro-ester B

Dihydro-Ester B (95 mg) was treated with 5% methanolic potassium hydroxide solution (5 cc) and the crude acid on esterification and chromatography afforded unchanged dihydro-Ester B (75 mg), m.p. and mixed m.p. 137–138°.

Attempted equilibration of dihydro-ester C

A similar treatment of dihydro-Ester C (82 mg) with 5% methanolic potassium hydroxide solution (5 cc) gave a crude acid, which on esterification followed by chromatography yielded unchanged dihydro-Ester C (60 mg), m.p. and mixed m.p. 150–151°.

Hydrolysis of dihydronimbin

Dihydroacid $C_{28}H_{32}O_8$. A solution of dihydronimbin (2·7 g) in 5% methanolic potassium hydroxide solution (150 cc) was refluxed for 1 hr, acidified with cold dil HCl, and extracted with ether. The ether solution was washed (water), dried (Na_2SO_4) and concentrated to ca. 50 cc. The solid on recrystallization from a mixture of acetone and hexane afforded the dihydroacid, $C_{28}H_{32}O_8$ (0·9 g),

m.p. 192–195°d, $[\alpha]_D$ + 229° (pyridine). (Found: C, 65·34; H, 6·84. $C_{28}H_{32}O_8$ requires: C, 65·20; H, 7·00%). Equiv. wt., Found (titration), 236. Calc., 230.

U.V. λ_{max} 208 mμ (E 10,800) and 292 mμ (E 49).

Desacetyl dihydronimbin

The above dihydroacid (1·6 g) was esterified with diazomethane and the crude ester (1·34 g), m.p. 144 146°, after crystallization from pet ether and ether afforded desacetyl dihydronimbin, m.p. 146–147°, $[\alpha]_D$ + 138° ($CHCl_3$). (Found: C, 66·70; H, 7·26. $C_{27}H_{34}O_8$ requires: C, 66·37; H, 7·43%).

U.V. λ_{max} 206 mμ (E 12,000) and 292 mμ (E 67).

I.R. ($CHCl_3$) Peaks at 3200 (hydroxyl), 1710 (saturated ester), 1700 (saturated ketone) and 1500 and 875 cm^{-1} (furan).

Acetylation of desacetyl dihydronimbin

Desacetyl dihydronimbin (0·3 g) was acetylated with pyridine (3 cc) and acetic anahydride (3 cc) and the crude product on crystallization from methanol afforded dihydronimbin, m.p. and mixed m.p. 214–215°. The infra-red spectra of this ester was identical with that of dihydronimbin.

Lithium aluminium hydride reduction of the mixture of esters A, B *and* C *and dehydrogenation of the product*

A solution of a gummy mixture of Esters A, B and C (0·95 g) in dry ether (50 cc) was added to a suspension of lithium aluminium hydride (1 g) in dry ether (150 cc). The reaction mixture was kept at 10° for 15 min, then refluxed for 5 hr, yielding a gummy material (0·86 g). This was heated with selenium powder (2·5 g) at 320–340° for 20 hr, and extracted with ether. The gummy residue (0·13 g) on chromatography over alumina and elution with pet ether gave an oil (0·06 g), the 1, 3, 5-trinitrobenzene adduct of which was found to be identical with that of 1, 2, 5-trimethylnaphthalene reported before.

Oxidation of ester B

Diketoester $C_{28}H_{30}O_6$. To a complex prepared[14] from pyridine (1 cc) and chromium trioxide (100 mg) was added a solution of Ester B (100 mg) in pyridine (1 cc) at 15°. The mixture was allowed to stand at room temp for ½ hr. The gummy product (100 mg) was chromatographed over alumina (10 g, deactivated with 0·6 cc of 10% aqueous acetic acid) and the solid (40 mg) eluted with pet ether and benzene (4:1), after crystallization from pet ether afforded the diketoester, m.p. 177°. (Found: C, 70·65; H, 7·09. $C_{28}H_{30}O_6$ requires: C, 70·40; H, 7·09%).

U.V. λ_{max} 206 mμ (E 14,000) and 309 mμ (E 123).

I.R. ($CHCl_3$) Peaks at 1710 (saturated ketone and ester), 1680 (α,β-unsaturated ketone) and 1500 and 875 cm^{-1} (furan).

Oxidation of desacetyl dihydronimbin

Diketodiester $C_{27}H_{34}O_8$. Desacetyl dihydronimbin (110 mg) in pyridine (1 cc) was similarly treated with a complex of chromium trioxide (100 mg) and pyridine (1 cc) yielding a gummy residue (100 mg) which on chromatography over alumina (12 g, deactivated with 0·4 cc of 10% aqueous acetic acid) and elution with pet ether and benzene (2:3) gave material (50 mg) which crystallized from pet ether and ether affording the diketodiester, m.p. 155–156°. (Found: C, 66·97; H, 6·66. $C_{27}H_{34}O_8$ requires: C, 66·65; H, 7·04%).

U.V. λ_{max} 206 mμ (E 14,800) and 304 mμ (E 104).

I.R. ($CHCl_3$) Peaks at 1735 (ketone and ester), 1500 and 872 cm^{-1} (furan).

'Pyronimbic acid'

'Pyronimbic acid' was prepared by the sublimation of nimbic acid[12] in vacuum, and crystallized from methanol, m.p. 265 267°d (reported[12] m.p. 263 267°), $[\alpha]_D$ +117° ($CHCl_3$). (Found: C, 72·84; H, 6·89. $C_{24}H_{28}O_5$ requires: C, 72·70; H, 7·12%). Mol. wt., Found (Rast), 384, (titration), 380. Calc., 396.

Similarly acids B and C on sublimation followed by crystallization afforded 'pyronimbic acid', m.p. and mixed m.p. 265 267°d.

Conversion of 'pyronimbic acid' to esters **B** *and* **C**

'Pyronimbic acid' (125 mg) was allowed to stand for ½ hr with 5% methanolic potassium hydroxide solution (2 cc), and acidified with cold dil HCl. The crude acid, on esterification followed by chromatography afforded Ester B (40 mg), m.p. and mixed m.p. 152-154° and Ester C (35 mg), m.p. and mixed m.p. 158 160°.

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