Phytochemistry, 1971, Vol. 10, pp. 857 to 864. Pergamon Press. Printed in England.

IDENTIFICATION OF NIMBIDIC ACID AND NIMBIDININ FROM *AZADIRACHTA INDICA**

C. R. MITRA, H. S. GARG and G.N. PANDEY†

Utilization Research Laboratory, National Botanic Gardens, Lucknow, U.P., India

(*Received* 25 *June* 1970)

Abstract—A new tetranortriterpene nimbidinin (II) from the neutral fraction of nimbidin, the amorphous bitter principle of *Azadirachta indica* seed kernel is shown to be biogenetically related to salannin. The crystalline acidic constituent nimbidic acid (I) has been found to be identical to salannic acid, derived from salannin.

A NUMBER of tetranortriterpenoids (C_{26}) have been isolated from different members of the Meliaceae[1] and are termed 'limonoids'. A well known plant of this family, *Azadirachta indica* A. Juss (syn. *Melia azadirachta*; nim) has been extensively studied and the principal crystalline bitter constituent nimbin has been found to be concentrated in the seed kernel.[2] In recent years a number of related principles, salannin,[3] azadirone, azadiradione and epoxyazadiradione,[4] melianone[5] and other related products have been reported from the Meliaceae.

When the main bulk of the amorphous bitter principle, nimbidin[6] obtained from the mother liquor of nimbin and nimbinin, is subjected to stepwise mild alkali (0·5–5%) wash and separated into the acidic and neutral fractions, two well defined constituents, nimbidic acid[6] from the acidic fraction and nimbidinin,[7] a new neutral tetranortriterpene, were obtained and they have now been further characterised and their structures elucidated.

The syrupy mother liquor of nimbidic acid (I), $C_{26}H_{34}O_7$ (P^+ 440, M^+ $-H_2O$), m.p. 228–30° on chromatography over silica gel yielded tiglic acid, m.p. 68–70° from the benzene eluted fraction while the benzene–chloroform (4:1) mixture yielded a fresh crop of nimbidic acid.

The i.r. spectra of nimbidic acid showed the presence of hydroxyl (3400 cm^{-1}), carboxyl carbonyl (1715 cm^{-1}) and oxide linkages (1080 and 1020 cm^{-1}). On methylation with diazomethane it yielded a methyl ester (Ia), $C_{27}H_{36}O_7$ (M^+ 472), m.p. 221–23°. Its NMR spectrum revealed the presence of only one carbomethoxy methyl at δ 3·6 ppm showing it

* Part III in a series on the constituents of *Azadirachta indica*; for Part-II, see C. R. MITRA, H. S. GARG and G. N. PANDEY, *Tetrahedron Letters* 2761 (1970).

† Present address: Department of Chemistry, Temple University, Philadelphia, U.S.A.

[1] G. P. MASS, *Planta Med.* Suppl. 86–96 (1966).

[2] C. R. NARAYANAN, R. V. PACHAPURKAR, S. K. PRADHAN, V. R. SHAH and N. S. NARASIMHAN, *J. Indian Chem. Soc.* **2**, 108 (1964); R. HENDERSON, R. MCCRINDLE, K. M. OVERTON, M. HARRIS and D. W. TURNER, *Proc. Chem. Soc.* 269 (1963).

[3] R. HENDERSON, R. MCCRINDLE, K. H. OVERTON and A. MELERA, *Tetrahedron Letters* 3969 (1964).

[4] D. LAVIE and M. K. JAIN, *Chem. Commun.* 178 (1967).

[5] D. LAVIE, M. K. JAIN and I. KIRSON, *J. Chem. Soc.* (C), 1347 (1967).

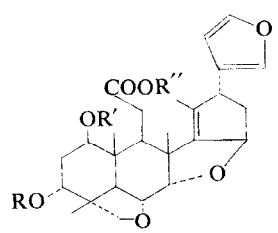
[6] S. SIDDIQUI and C. R. MITRA, *J. Sci. Ind. Res., India* **4**, 5 (1945).

[7] C. R. MITRA, *Neem Monograph, Ind. Cent. Oil Seeds Committee, Hyderabad, India*, pp. 84–93 (1963).

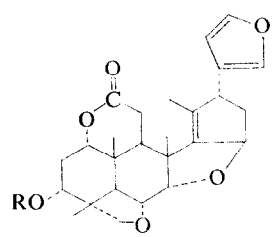
to be a monocarboxylic acid. The methyl ester on acetylation (Py/Ac_2O) yielded a diacetate ester (Ib), $C_{31}H_{40}O_9$ (M^+ 556), m.p. 235–37°. The NMR spectrum of Ib showed the presence of one methoxyl methyl at δ 3·35 ppm and two acetyl methyls at δ 2·08 and 2·14 ppm. The NMR spectrum of Ib and other physicochemical properties of nimbidic acid were found to be identical with those of salannic acid,[8] a hydrolysis product of salannin[3] reported from *Melia dubia*. Nimbidic acid (I) on acetylation (Py/Ac_2O) afforded a hitherto unreported lactone acetate, 3-acetyl nimbidic acid-1,(12)-lactone (Ic), $C_{28}H_{34}O_7$, (M^+ 482), m.p. 226–28°. This spontaneous tendency of nimbidic acid to lactonise is in agreement with the absence of the molecular ion peak (M^+ 482) in the mass spectra of nimbidic acid itself, which showed the parent ion peak at *m/e* 440 (M^+-H_2O). Since the ester showed the molecular ion peak at *m/e* 472, the loss of water molecule in I must be due to lactonisation and not from the elimination of the hydroxyl. These properties of nimbidic acid lead to its identity with salannic acid.

The neutral crystalline component, nimbidinin (II) $C_{26}H_{34}O_6$ (M^+ 442), m.p. 282–84° was further purified through crystallisation and chromatography. It showed u.v. maximum at 225 nm. The i.r. spectrum of nimbidinin revealed the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1708 cm^{-1}) groups besides the presence of a trisubstituted double bond (820 cm^{-1}) and ether linkage (1160 and 1070 cm^{-1}). Indication of an ester grouping was apparently absent from the i.r. spectra.

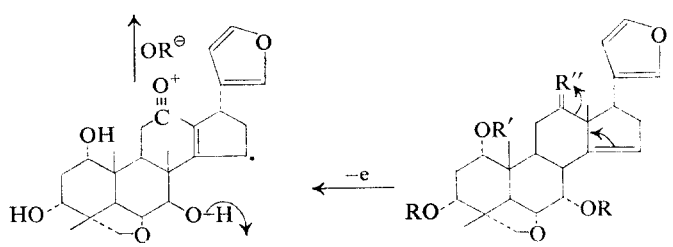
On acetylation (Py/Ac_2O) nimbidinin yielded a triacetate (IIa) $C_{32}H_{40}O_9$ (M^+ 568), m.p. 222–24° showing the presence of three hydroxyl groups in the molecule. It yielded a



I R=R′=R″= H
Ia R=R′= H, R″= CH_3
Ib R=R′= Ac, R″= CH_3
Id R= Ac, R′= Tg, R″= CH_3



Ic R= Ac



Scheme I.

II R= R′= H, R″= O
IIa R= R′= Ac, R″= O
IIb R=R′= H, R″= NOH
IIc R=Ac, R′= Tg., R″= O

[8] L. B. DE SILVA, W. STOCKLIN and J. A. GEISMAN, *Phytochem.* **8**, 1817 (1969).

monooxime (IIb), $C_{26}H_{35}O_6N$, m.p. 274–76° showing the presence of only one carbonyl group. The NMR spectrum of the nimbidinin triacetate (IIa) did not show the presence of any aldehydic proton and as such the carbonyl group must be present as a six membered cyclic ketone. The NMR and mass spectral studies of nimbidinin and its acetate supported its structure II and on comparison with that of salannin diacetate[3] (Ib) and nimbin[2] (III) distinguished it from them.

The NMR spectra of salannin diacetate and nimbidinin triacetate are compared in Table 1. The NMR spectrum of IIa showed a few dissimilarities with those of salannin diacetate. Salannin diacetate showed the presence of three tertiary and one vinylic methyl groups while nimbidinin triacetate showed the presence of four tertiary methyl groups and absence of vinylic methyl group. Thus the double bond in the latter must not be Δ^{13} (also the i.r. spectra show it to be trisubstituted rather than tetrasubstituted Δ^{13}).

TABLE 1. NMR PROTON SIGNALS IN δ (ppm)

Protons	Salannin	Nimbidinin triacetate
Quaternary methyl	1·0, 1·23 and 1·33 (3 × CH_3)	1·0, 1·02, 1·17 (6 H) (4 × CH_3)
Vinylic methyl	1·69 (1 × CH_3)	— —
Acetate	1·95 (1 × Ac)	1·99, 2·0 and 2·02 (3 × Ac)
Ester methyl	3·28	— —
H – 1	4·95	4·97
H – 3	4·80	4·70
H – 5	2·78	2·7
H – 6	4·0	4·20
H – 7	4·18	5·6 (shift due to acetate)
β–furan	6·33 (H) and 7·25 (2 H)	6·65 (H) and 7·45 (2 H)
C–23 methylene	3·75, 3·58	3·55

There are only two acetyl groups in salannin acetate while nimbidinin acetate showed three acetyl groups. Since the NMR spectrum of nimbidinin triacetate manifested the presence of two diffused triplets at δ 4·7 and 4·97 ppm identical to those observed in case of salannin diacetate, which are individually coupled with C-2 methylene protons appearing at δ 2·4 ppm ($J_{ax_2} = J_{bx_2} = 2{\cdot}5$ Hz), two of the three hydroxyls in nimbidinin would therefore be at C-1 and C-3 similar to those in salannic acid.

The other remarkable features in the NMR spectrum of nimbidinin acetate are the C-5, C-6, C-7 carbon chain hydrogens resembling those of salannin and nimbin (*loc. cit.*). H-6 proton (double doublet centred at δ 4·24 ppm) is coupled to two neighbouring protons H-5 (J = 11 Hz) and H-7 (J = 2·5 Hz). The H-7 proton in turn appears at δ 5·6 ppm as a doublet (J = 2·5 Hz). These observations confirm the configuration of the protons attached at C-5, C-6 and C-7 as axial, axial and equitorial and the absence of protons at C-8 and C-10 and the location of oxygen functions at C-6 and C-7. Further, the presence of a methylene group adjacent to an oxide link was observed as two proton signal at δ 3·55 ppm indicating the oxide link in nimbidinin to be through C-23 and C-6 as found in salannin (*loc. cit.*). Thus the third hydroxyl group in nimbidinin can be placed at C-7.

The presence of a β-substituted furan ring attached to C-17, a common feature of meliacins, in nimbidinin, was evident from the NMR signals at δ 6·62 (1H) and δ 7·42 (2H) ppm. Thus all the six oxygen atoms in nimbidinin are accounted for by three hydroxyls, one carbonyl, one furan and an oxide link. Evidently nimbidinin (II) does not have an oxide link at C-7 (15) and instead, the oxide ring is open having the additional hydroxyl group at C-7 and a double bond at C-14 (15) confirmed by the olefinic proton at δ 5·7 ppm. Taking into account its molecular weight, the presence of carbonyl as a cyclic ketone, and a double bond, the ring-C in nimbidinin must be closed, in contrast to that in salannin and nimbin. This fact was further supported by the absence of vinylic methyl, a common feature in this series of compounds.

The carbonyl frequency (i.r.) observed in nimbidinin is in agreement with a six rather than a five membered cyclic ketone. Since the spectral data show that the carbonyl cannot be in ring-D, it can only be placed at C-11 or C-12. The optical rotatory dispersion curve of nimbidinin shows a positive Cotton effect (peak at $(\alpha)_{267}$–14·4° and trough at $(\alpha)_{246}$–23·2°) and is in agreement with C-11 and/or C-12 carbonyl sterols.[9] Since no sharp signals appeared in the NMR spectrum for the protons *alpha* to the carbonyl in the region δ 2–3 ppm, the presence of the carbonyl at C-12 is more probable as the placement of the carbonyl at C-11 would deshield the protons at C-9 as well as at C-12, while with C-12 carbonyl these signals should be like that for any C-3 carbonyl group. Further, the placement of the carbonyl at C-12 is more logical from its probable biogenesis to give rise to nimbidic acid (I) *vis-à-vis* salannin (Id) on further oxidation as shown in Scheme I.

Thus nimbidinin (II) appears to be an intermediatory product of salannin (Id). This is supported by the co-occurrence of nimbidinin (II) and nimbidic acid (I), the acid related to salannin (Id), in *Azadirachta indica*.

Since nimbidinin is obtained by step-wise mild alkaline hydrolysis of the amorphous bitter principle, the possibility of the presence of a tiglate and acetate at C-1 and C-3 respectively in the parent natural product (representing IIc) yielding nimbidinin (II), as in salannin (Id), is not ruled out since tiglic acid was obtained from the acidic fraction of the hydrolysate.

The structures of nimbidinin and nimbidic acid were further supported by comparative mass spectral studies with these compounds and those in nimbin (III) and the significant mass peaks of these compounds are tabulated in Table 2.

The presence of C-17 β-substituted furan ring in all the three compounds gave a fragment a, *m/e* 81. In case of nimbidinin (II) there appeared an additional fragment at *m/e* 95 constituting of the fragment b, arising out of the rupture of 15:16 bond and it may be

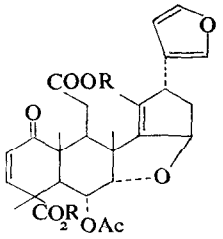
TABLE 2. MASS SPECTRAL DATA

Compounds	Mass fragments (*m/e*)
Methyl nimbidate (Ia)	472 (M^+) (base peak), 441 (M^+-MeO), 399 (M^+-CH_2-$COOCH_3$), 381 (399-H_2O), 341, 282, 259, 244, 231, 215, 202 (d), 185 (g), 173, 81(a)
Nimbidinin (II)	442 (M^+) (base peak), 427 (M^+-CH_3; i), 377, 359, 333 (j), 315 (j)-H_2O), 227 (e), 213 (h), 95 (b), 81 (a)
Nimbin (III)	540 (M^+), 509 (M^+-MeOH), 498 (M^+-CH_2CO), 480 (M^+-AcOH), 421 (480-CO_2Me), 389, 383, 340 (f), 273 (c), 231 (273-CH_2CO) (base peak), 174, 105, 81 (a)

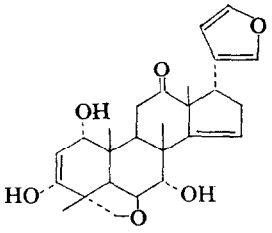
[9] C. DJERASSI, *Optical Rotatory Dispersion*, p. 44, McGraw-Hill, New York (1960).

attributed to the shift in the double bond in ring-D and closure of ring-C in nimbidinin. This also confirmed the absence of carbonyl group in ring-D in nimbidinin.

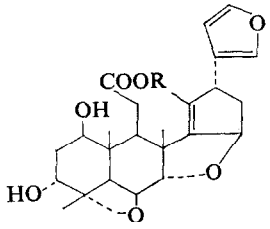
The cracking of ring-B due to the presence of oxygen functions at C-6 and C-7 gave rise to fragment c, *m/e* 273 in case of nimbin (III), fragment d, *m/e* 202 in case of methyl nimbidate (Ia), while nimbidinin (II), due to the closed ring-C, instead gave rise to the fragment e, *m/e* 227. The presence of a double bond in ring-A in nimbin, yielded a fragment f, *m/e* 340 by RDA fragmentation followed by the loss of carbomethoxy group at C-11.



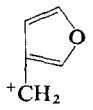
III R = CH_3



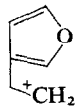
II



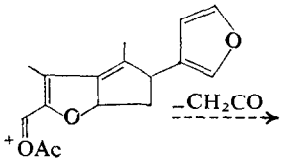
I R = H
Ia R = CH_3



a (*m/e* 81)

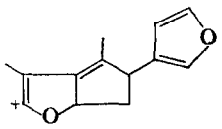


b (*m/e* 95)

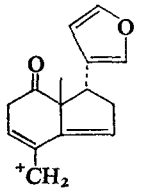


m/e 231

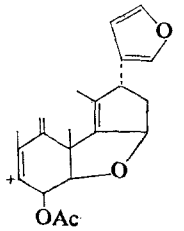
c (*m/e* 273)



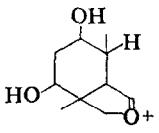
d (*m/e* 202)



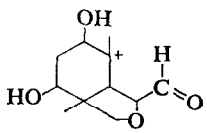
e (*m/e* 227)



f (*m/e* 340)



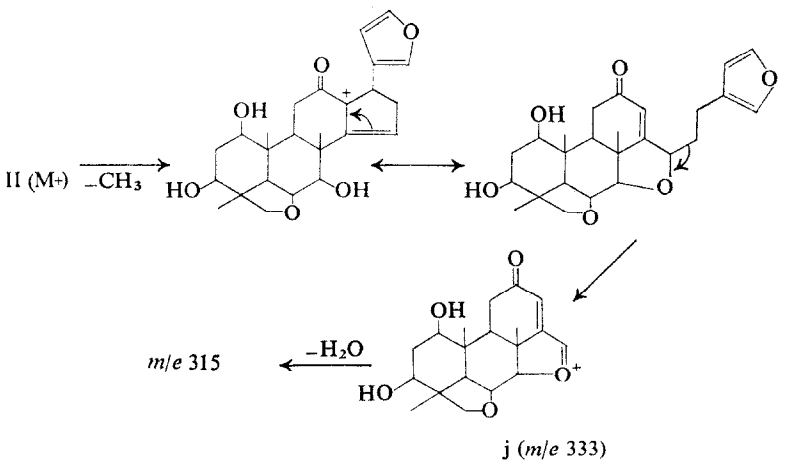
g (*m/e* 185)



h (*m/e* 213)

In case of methyl nimbidate there is a prominent peak at *m/e* 185 which could have arisen by the opening of 9:10 bond with a hydrogen transfer to C-10 accompanied by simultaneous cracking of 6:7 bond facilitated by the presence of the C-6 (23) oxide linkage to yield fragment g, *m/e* 185; the fragment g was not evident in nimbin owing to the absence of this C-6 (23) oxide link. In case of nimbidinin, however, the presence of C-7 hydroxyl group instead of the C-7 (15) oxide link, facilitates to generate fragment h, *m/e* 213 and a weak peak *m/e* 185.

The other significant fragmentation of nimbidinin was the appearance of an ion *m/e* 333 which may be shown to arise by the loss of C-13 methyl from the molecular ion to give fragment i, *m/e* 427; the latter in turn may loose the furan ring along with 16 and 17 carbon atoms followed by closure of C-7 (15) oxide ring to yield the fragment j, *m/e* 333 as shown in scheme below. The fragment j with the loss of a molecule of water from the hydroxyl groups, gives rise to the peak at *m/e* 315 (confirmed by the metastable ion at *m/e* 298·4, calcd. 297·97 *m/e*).



Thus the mass spectral fragmentation pattern of nimbidinin was in agreement with its structure (II) which distinguishes it from the skeletal configuration of both nimbin (III) and methyl nimbidate (Ia) *vis-à-vis* salannin (Id).

The other common features corresponding to the loss of methanol and acetic acid molecules from the esters and acetate groupings in case of nimbin and from the ester groupings in case of methyl nimbidate were absent in case of nimbidinin. The base ion peak appeared at *m/e* 231 in case of nimbin which may be shown to arise by the loss of CH_2CO radical from fragment c, *m/e* 273 (confirmed by the metastable peak at *m/e* 195·4; calcd. 195·46 *m/e*). In the other two cases, it was the respective molecular ion peak which appeared as the base ion peak.

Thus the mass spectral studies with nimbin series of compounds with special reference to nimbin, nimbidinin and methyl nimbidate (salannic acid methyl ester)[8] reveal the considerable influence of the oxide links in ring-B and that of a closed or open ring-C, on their mass spectral fragmentation.

In case of nimbin (III) the oxide link is through C-7 (15) with the C-6 acetyl group and in that of nimbidinin (II) it is through C-6 (23) with C-7 hydroxyl group while in nimbidic

acid (I) there are two oxide links through C-6 (23) and C-7 (15). Further in case of nimbidinin (II) ring-C is a closed one while in the other two cases it is open having the C-12 carbomethoxy group.

EXPERIMENTAL

M.ps were taken in open capillaries and are uncorrected. The i.r. spectra were recorded in KBr films and NMR spectra were run in $CDCl_3$ using TMS as internal reference.

Isolation of Nimbidic Acid and Nimbidinin

The total amorphous bitter fraction of *A. indica*, nimbidin[1] obtained from the mother liquor of nimbin and nimbinin was stepwise treated with (0·5–5% alc. KOH).[10] The alkali-soluble fraction was made mildly acidic and processed to yield nimbidic acid, $C_{26}H_{34}O_7$, m.p. 228–30°. I.r. bands at 3400, 2900, 1715, 1460, 1350, 1200, 1180, 1060, 1040, 960, 880 and 790 cm^{-1}. Mass: 440 (P^+; M^+-H_2O), 422 (W), 245, 200, 185, 173 and 146 etc. *m/e*. (Found: C, 68·14, H, 7·69; $C_{26}H_{34}O_7$ requires: C, 68·12, H, 7·42%.)

The neutral bitter fraction left after treatment with 5% alkali was purified through repeated chromatography and crystallisation when it yielded pure nimbidinin (single spot, TLC), $C_{26}H_{34}O_6$, m.p. 282–84°. I.r. bands at 3400, 2900, 1708, 1460, 1340, 1160, 1070, 1020 and 820 cm^{-1}. ORD: $(\alpha)_{230}$ +1·40, $(\alpha)_{246}$-5·5°, $(\alpha)_{267}$-3·20, $(\alpha)_{292}$-3·65 and $(\alpha)_{400}\pm0$.

(Found: C, 71·38; H, 8·52, $C_{26}H_{34}O_6$ requires: C, 70·59, H, 7·69%.)

Methyl Nimbidate

Nimbidic acid (250 mg) in MeOH (10 ml) was treated with freshly prepared ethereal solution of diazomethane in excess in cold (< 5°) and left at room temperature overnight. The residue on recrystallisation (alc.) yielded (250 mg) methyl nimbidate, $C_{27}H_{36}O_7$ (M^+ 472), m.p. 221–23°.
I.r. 3360, 2900, 1725, 1460, 1390, 1320, 1300, 1250, 1200, 1160, 1070, 1040, 1000, 950, 890, 840 and 790 cm^{-1}.
NMR: δ 0·90 (3H); 1·10 (3H), 1·28 (3H), 1·72 (3H), 2·13 (2H), 2·45 (2H), 2·70 (d.d, J=6 Hz, H), 3·60 (3H), 3·8–4·1 (4H), 4·25 (H), 4·55 (H), 5·5 (m, H), 6·2 (H) and 7·35 (2H) ppm.

(Found: C, 68·73, H, 7·90, $C_{27}H_{36}O_7$ requires: C, 68·55, H, 7·62%.)

Methyl Nimbidate Diacetate

Methyl nimbidate (150 mg) was left with pyridine (5 ml) and Ac_2O (1·5 ml) at room temp. for 24 hr. Poured over crushed ice, washed acid free and chromatographed over silicagel, eluted with C_6H_6–$CHCl_3$ (1:2) yielding methyl nimbidate diacetate, $C_{31}H_{40}O_9$ (M^+ 556), m.p. 235–37°. I.r. bands at 2900, 1725, 1450, 1390, 1315, 1250, 1200, 1150, 1070, 950, 890 and 790 cm^{-1}. NMR: δ 0·98 (3H), 1·20 (3H), 1·31 (3H), 1·66 (3H), 2·06 (3H), 2·13 (6H), 2·4–2·2 (3H), 2·75 (2H d.d, J=6 Hz), 3·32 (3H, 3·65 (2H), 4·15 (H) (t, J=2·5 Hz), 4·80 (1H, t, J=2·5 Hz), 4·95 (H, t, J=2·5 Hz), 5·54 (m, H), 6·35 (H), 7·35 (2H) ppm. (Found: C, 66·47; H, 7·34, $C_{31}H_{40}O_9$ requires: C, 66·90; H, 7·19%.)

Acetylation of Nimbidic Acid: 3-*Acetyl Nimbidic Acid*-1 (12) *Lactone* (*Ic*)

Nimbidic acid (200 mg) in pyridine (5 ml) left overnight at room temp. with Ac_2O (1·5 ml) and processed as above, to yield Ic, $C_{28}H_{34}O_7$ (M^+ 482), m.p. 226–28°. I.r. bands at 2900, 1750, 1460, 1390, 1250, 1180, 1080, 1040, 950, 880, 795 and 780 cm^{-1}. Mass: 482 (M^+), 467 (W), 422 (W), 417, 245, 244, 227, 202, 201, 200, 185, 173, 147, 133 and 81 *m/e*. (Found: C, 69·90, H, 7·39, $C_{28}H_{34}O_7$ requires: C, 69·70, H, 7·05%.)

Nimbidinin Triacetate (*IIa*)

Nimbidinin (200 mg) in pyridine (5 ml) and Ac_2O (2 ml) was left overnight at room temp. and then refluxed at 120° for 2 hr and poured onto crushed ice. The residue on recrystallization (alc.) yielded fine needles (200 mg) of Nimbidinin triacetate, $C_{32}H_{40}O_9$ (M^+ 568). m.p. 222–24°. I.r.: 2900, 1720, 1460, 1390, 1250, 1160, 1070, 1040, 1030, 950, 890, 820 and 790 cm^{-1}. (Found: C, 67·28; H, 7·54, $C_{32}H_{40}O_9$ requires: C, 67·60; H, 7·04%.)

Nimbidinin Mono-oxime

Nimbidinin (100 mg) in alcohol (10 ml) and pyridine (2 ml) was refluxed with NH_2OH–HCl (200 mg) on water bath for 12 hr. The product was crystallised (alc.) to yield Nimbidinin oxime, $C_{26}H_{35}O_6N$, m.p. 274–76°. I.r.: 3400, 2900, 1460, 1390, 1160, 1070, 1030, 940, 890 and 810 cm^{-1}. (Found: C, 68·05; H, 7·95; N, 2·59, $C_{26}H_{35}O_6N$ requires: C, 68·27; H, 7·65; N, 3·05%.)

[10] C. R. MITRA, Investigation on some Indigenous Medicinal oils, Ph.D. Thesis, University of Poona, India, p. 47 (1957).

Acknowledgements—The authors thank Dr. G. Snatzke and Dr. H.-W. Fehlhaber of the University of Bonn for certain mass and NMR spectra and discussion thereof during the stay of one of us (CRM) in Bonn under the DAAD Exchange Programme to whom also the thanks are due. Thanks are also due to Dr. K. G. Dass, Dr. Nitya Nand and Prof. W. Klyne for m.s., i.r., NMR spectra and ORD curve, J. Saran for microanalysis. This work was partly supported by the U.S.D.A. PL-480 Funds Grant No. FG-IN-322.