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ALKALOIDS OF *MICHELIA LANUGINOSA* WALL

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Abstract—Along with the oxoaporphine alkaloid liriodenine, a new oxoaporphine alkaloid lanuginosine and a new noraporphine alkaloid michelanugine have been isolated from the trunk bark of *Michelia lanuginosa*. They have been shown to possess 1,2-methylenedioxy-9-methoxy-7-oxoaporphine and 1,2-methylenedioxy-9-methoxy-7-hydroxynoraporphine structures through chemical and spectral studies. The stereostructure of michelanugine (6a-H as β and 7-OH as α) has been elaborated with the help of PMR and rotational data.

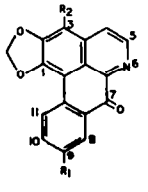
Michelia lanuginosa Wall has recently been shown to produce[1,2] a number of interesting sesquiterpene lactones of germacrane type. Investigations on the alkaloidal constituents of the same plant have resulted in the isolation of three bases. Their structures and the stereochemistry (of one of them) have been elaborated on the basis of physical and chemical studies.

The major alkaloid, crystallising from $CHCl_3$ in yellow needles, analysing for $C_{17}H_9O_3N$ (M^+ 275), m.p. 282°(d), formed deep red solutions in mineral acids and exhibited a green fluorescence in $CHCl_3$ solution. It possesses a methylenedioxy group and a highly conjugated CO function (IR). Its UV spectrum is characteristics of an oxoaporphine chromophore. This compound has subsequently been identified as liriodenine[3] (**1**).

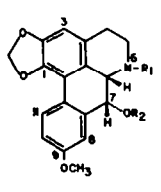
The orange-yellow alkaloid (**2**) designated lanuginosine, analysing for $C_{18}H_{11}O_4N$ (M^+ 305), m.p. 317–19°(d), crystallised from $CHCl_3$ as transparent glistening orange red needles, which tarnished to orange yellow in course of time. It formed deep red solutions in mineral acids and exhibited deep green fluorescence in $CHCl_3$ solution. Its IR spectrum indicated the presence of a methylenedioxy and a highly conjugated CO function. The UV spectrum was similar to that of its congener liriodenine. The presence of one OMe group was evident by the Zeisel–Viebock method. The non-identity of lanuginosine with atherospermidine (**3**)[4] and the appearance of the methylenedioxy protons and H-3 signals as singlets in the same region as those in liriodenine and atherosperimidine indicated the absence of the OMe group in ring A. The appearance of the lowest field proton as doublet (coupling with an adjacent proton) in the PMR spectrum (60 MHz, CF_3COOH) run in a JEOL machine suggested[5] lanuginosine to have 9-methoxyliriodenine structure (**2**). Conclusive chemical support to this structure (**2**) was received by its conversion to (±)xylopine acetate through reduction of **2** with Zn/HCl in HOAc followed by acetylation and also by its unequivocal synthesis by Govindachari *et al.*[6] The synthetic and natural specimens were found to be identical by direct comparison.

In an attempt to establish the structure through decoupling experiments the PMR spectrum of **2** was taken in an XL-100 machine (CF_3COOH solvent). The resolved spectrum showing signals at δ 4·06 (C_9–OCH_3), 6·59 (methylenedioxy), 7·48 (H-3), 7·60 (dd, J = 9 Hz and 3 Hz, H-10), 8·01 (d, J = 3 Hz, H-8), 8·42 (d, J = 6 Hz, H-4), 8·72 (d, J = 6 Hz, H-5) and 8·72 ppm (d, J = 9 Hz, H-11) is fully in accord with the assigned structure **2**. This new spectrum discards the proton signals (JEOL) in the aromatic region reported earlier.[5] The earlier spectrum showed a gradual increasing shift of the aromatic proton signals towards the lowfield, the position of the H-11 signal being at 0·31 ppm lower field. An alkaloid with the same physical and chemical properties and structure as that of lanuginosine was isolated from *Stephania abyssinica*, and named oxoxylopine by Kupchan *et al.*[7] prior to any direct comparison with lanuginosine. Subsequently they were shown to be identical.[6,8] Furthermore, lanuginosine differed significantly from the synthetic 10-methoxy isomer[6] (the suggested structure of lanuginosine by Kupchan *et al.*[7]) in their physical and spectral properties. Casagrande *et al.*[9] recently isolated lanuginosine from *Xylopia brasiliensis* and synthesised it. The name lanuginosine, being first published, has been retained in literature.[10] The mass spectrum of lanuginosine indicated no ready skeletal fragmentation in accord with its completely aromatic structure. The only other significant peak at *m/e* 275 was assigned to the M^+–CH_2O fragment.

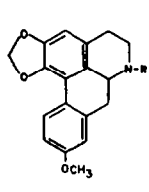
Besides lanuginosine and liriodenine, another new secondary base, michelanugine (**4**) has been isolated in an amorphous state in very poor yield from the trunk bark of *M. lanuginosa*. It formed crystalline derivatives, viz hydrochloride $C_{18}H_{17}O_4N$, HCl (M^+ 311), m.p. 275°(d), $[\alpha]_D$ −105° (EtOH) and a nonbasic O,N-diacetylderivative (**5**) $C_{22}H_{21}O_6N$ (M^+ 395), m.p. 256°, $[\alpha]_D$ −503° ($CHCl_3$). **5** was found to contain OMe (Zeisel) and methylenedioxy (Labat) groups. Michelanugine showed UV absorption maxima typical of nonphenolic aporphine alkaloids and closely similar to that of xylopine (**6**).[11] The UV spectrum of **5** also resembled that of N-acetylxylopine (**7**). This suggested that the oxygen functions in michelanugine were located at 1, 2 and 9 positions of the aporphine nucleus (cf xylopine). The IR spectrum (KBr) of michelanugine hydrochloride showed the presence of OH and/or NH functions and methylenedioxy group; that of **5** showed bands for



1: $R_1 = R_2 = H$
2: $R_1 = OCH_3$, $R_2 = H$
3: $R_1 = H$, $R_2 = OCH_3$



4: $R_1 = R_2 = H$
5: $R_1 = R_2 = COCH_3$



6: R = H
7: R = $COCH_3$

methylenedioxy, OAc and NAc functions, but no band for OH or NH functions were present. Insolubility of michelanugine in aqueous alkali revealed the nonphenolic nature of the OH group. CrO_3–Py oxidation of michelanugine afforded lanuginosine (2) in very poor yield and reduction under Clemmensen condition furnished (−)-xylopine (in low optical yield) characterised as its acetate (7).

The above conversions conclusively established the structure of michelanugine as 7-hydroxyxylopine (4). The PMR spectra of 4 and 5 are also in agreement with their proposed structures. The small coupling constant (3 Hz) of the H-6a and H-7 in the PMR spectrum (100 MHz) of 5 indicated their *cis*-relationship [Decoupling of the δ 5·41 ppm signal (J = 3 Hz) collapses the δ 6·22 ppm signal (J = 3 Hz) to a singlet]. Since both michelanugine, its O,N-diacetylderivative and the derived N-acetylxylopine are laevorotatory it is suggested that H-6 in this alkaloid should be β-oriented[12] and hence the OH at C-7 is α-oriented. The mass spectral fragmentation behaviour of 5 and hydrochloride of 4 were typical of aporphines[13] and fully consistent with their structures.

EXPERIMENTAL

All m.ps were determined in open capillaries and are uncorrected. The UV spectra were measured in aldehyde free ethanol soln in a Carl Zeiss Spectrophotometer Model VSU-1 (manual). IR spectra were examined in KBr pellets with a Perkin Elmer IR Grating Spectrophotometer. PMR spectra were measured in Varian A60D machine, unless otherwise mentioned, using TMS as internal standard. The rotations were measured in Hilger–Watts M-511 Microoptic Photoelectric Polarimeter. Mass spectra were run in an A.E.I. MS-9 mass spectrometer operating at 70 e.v. and using direct insertion probe. TLC experiments were done with SiO_2 gel G. adsorbent and $CHCl_3$:MeOH (93:7) mixture as developer.

Isolation of liriodenine (1) *and lanuginosine* (2). Defatted milled trunk bark (1·3 kg) of *M. lanuginosa* was exhaustively extracted with $CHCl_3$ (using a Soxhlet extractor) and then with EtOH (by cold percolation). The basic fraction from the $CHCl_3$ extract was chromatographed over Brockmann alumina (Neutral Grade I, 80 g, 32 cm × 1·8 cm). The yellow band was eluted out with $CHCl_3$. The residue left on removal of the solvent showed the presence of two close spots in TLC when viewed in UV light (R_f 0·61 and 0·58). The constituents could not be separated by usual chromatography procedure. Repeated fractional crystallisations from $CHCl_3$ afforded a less soluble fraction in pure state which was finally purified from adhering gummy matters by chromatography over alumina to yield lanuginosine (2) (80 mg) as glistening orange-red needles (tarnishing to orange yellow in course of time), m.p. 317–19°(d), R_f 0·61 (orange fluorescence in UV light), formed red solns in mineral acids and exhibited a deep green fluorescence in $CHCl_3$ soln. It shows λ_{max} (EtOH) 246 nm (log ϵ 4·54), 271 (4·44), 315 (3·89) undergoing bathochromic shift in acid medium: λ_{max} (EtOH–HCl) 258 nm (4·57), 283 (4·47), 334 (3·83); ν_{max} (KBr) 1660 (conjugated C=O), 1490, 1416, 1355, 1257, 1125, 1040, 955 and 940 cm^{-1} (CH_2 ); *m/e* 305 (M^+, 100%), 275 (M^+–CH_2O, 30%). (Found: C, 70·3; H, 3·58; OCH_3, 9·80; N, 4·48. $C_{18}H_{11}O_4N$ requires: C, 70·8; H, 3·60; N, 4·60; OCH_3, 10·1%).

From the mother liquor after removal of lanuginosine the major yellow oxoaporphine (170 mg), m.p. 282°(d), R_f 0·58 was isolated as the more soluble component and finally by chromatography over alumina. It showed yellow fluorescence in UV light; λ_{max} (EtOH) 247·5 nm (log ϵ 4·31), 269 (4·21), 303 (3·74); λ_{max} (EtOH–HCl) 257 nm (4·41), 280 (4·30), 334 (3·76); ν_{max} (KBr) 1660 (conjugated CO), 1495, 1420, 1357, 1255, 1120, 1035, 958 and 942 cm^{-1} (CH_2 ); in PMR (CF_3COOH): δ 7·63 (3-H), 6·72 ppm (CH_2 ). The compound was identified as liriodenine by direct comparison through m.m.p., TLC, IR, UV.

Isolation of michelanugine (4). The basic fraction from the alcoholic extract was chromatographed over Brockmann alumina (80 g, 24 × 2·2 cm). The yellow band was eluted with $CHCl_3$ and the residue from these eluates afforded a further quantity of liriodenine (5 mg) and lanuginosine (50 mg). The residue from $CHCl_3$:MeOH (19:1) eluted fraction after repeated chromatography over alumina afforded an amorphous base (110 mg) designated michelanugine having R_f 0·34.

Michelanugine hydrochloride. To a soln of michelanugine (110 mg) in abs EtOH (0·5 ml), a microdrop of A.R. HCl was added. The soln was cooled in ice and the mother liquor was decanted off the separated residue which was crystallised twice from abs EtOH to get pure michelanugine hydrochloride (72 mg), m.p. 275°(d), $[\alpha]_D$ −105° (EtOH, c 0·62); λ_{max} 217 nm (log ϵ 4·49), 237 (infl 4·16), 279 (4·29) and 322 (infl 3·62), unchanged by the addition of acid or alkali; IR: 3300 (NH and OH), 1500, 1405, 1375, 1250, 1125, 1042, 960 and 935 cm^{-1} (CH_2); PMR (CF_3COOH): δ 3·60 (9-OCH_3), 3·92 (m, 6a-H), 4·84 (bs, 7-H), 5·62 and 5·75 (bs each, CH_2  , 1,2), 5·87 (7-OH), 6·27 (3-H), 6·73 (d, 3 Hz, 8-H), 6·78 (dd, 3 and 9·5 Hz, 10-H) and 7·87 ppm (d, 9·5 Hz, 11-H); mass: *m/e* 311 (M^+, 100%), 310 (M^+–H, 100%; m* 309), 293 (M–H_2O, 20%, m* 276·04), 282 (M^+–NH=CH_2, 36%), 281 (*m/e* 282-H, 40%, m* 280), 252 (*m/e* 282-CH_2O, 15%, m* 225·2), 224 (*m/e* 252-CO, 15%). (Found: C, 59·9; H, 5·34; N, 3·82; OCH_3, 8·51. Calc. for $C_{18}H_{17}O_4N$, HCl: C, 60·4; H, 5·31; N, 3·90; OCH_3, 8·70%).

O,N-*Diacetylmichelanugine* (5). The residue from the alcoholic mother liquor obtained during the preparation of michelanugine hydrochloride was treated with Ac_2O (0·5 ml) and fused NaOAc (50 mg) and left overnight. Usual work up followed by chromatography over alumina afforded O,N-diacetylmichelanugine crystallising from chloroform-petrol as fine needles (40 mg), m.p. 256°, $[\alpha]_D$ −503° ($CHCl_3$, *c* 0·46); R_f 0·77; λ_{max} 217 nm (log ϵ 4·53), 276 (4·26), 283 (infl 4·22) and 3·22 (sh 3·68); IR: 1725, 1245 (C–O–CO–), 1635 (amide CO), 1500, 1420, 1366, 1245, 1125, 1055, 955 and 935 cm^{-1} (CH_2 ); PMR (δ, 100 MHz, $CDCl_3$): 1·87 ($NCOCH_3$), 2·21 (7-$OCOCH_3$), 3·83 (9-OCH_3), 5·41 (d, 3 Hz, 6a-H), 6·05 and 6·08 (each 1H, d, 10 Hz, CH_2  , 1,2), 6·22 (d, 3 Hz, 7-H), 6·59 (3-H), 6·97 (dd, 3 and 9 Hz, 10-H), 7·13 (d, 3 Hz, 8-H) and 8·12 ppm (d, 9 Hz, 11-H); mass: *m/e* 395 (M^+, 0·5%), 353 (M^+–CH_2CO, 0·5%), 352 (M^+–CH_3CO, 1%), 335 (M^+–HOAc, 59%), 322 (*m/e* 352-CH_2O, 4%, m* 294·5), 310 (*m/e* 353-CH_2CO, 8%), 305 (*m/e* 335-CH_2O, 100%, m* 277·7), 293 (*m/e*, 353-HOAc, 35%), 281 (*m/e* 310-NH=CH_2, 10%), 280 (*m/e* 281-H or *m/e* 310-CH_2O, 23%), 263 (*m/e* 293-CH_2O, m* 236·1 or *m/e* 280 + H − H_2O, 64%), 251 (*m/e* 280-NH=CH_2, 13%). (Found: C, 67·1; H, 5·28; N, 3·58; OCH_3, 7·82. $C_{22}H_{21}NO_6$ requires: C, 66·8; H, 5·34; N, 3·54; OCH_3, 7·84%). The same compound was obtained on acetylation of the hydrochloride in the similar way.

Clemmensen reduction of lanuginosine (2) *to xylopine* (6). A soln of lanuginosine (60 mg) in AcOH acid–water (2:1, 6 ml) was treated with powdered zinc (4 g) and conc HCl (6 ml). The mixture

was heated on a water bath till all the Zn had been consumed and to the mixture, which was still red indicating the presence of unreduced oxoaporphine, was added additional Zn dust (2 g) and conc HCl (6 ml); the mixture was then warmed on a water bath till all the Zn had been consumed and the soln became colourless. The cooled acidic soln was strongly basified using a large excess of strong NH_3 soln and was extracted with $CHCl_3$ (4 × 150 ml). The combined $CHCl_3$ extract after being washed and dried was evaporated to give a crude material (50 mg), R_f 0·35 showing the same UV absorption maxima and same TLC behaviour as xylopine. The material, which resisted crystallisation, was treated with Ac_2O (1 ml) and fused NaOAc (50 mg) and left overnight. The mixture was treated with water, neutralised with $NaHCO_3$ and extracted with $CHCl_3$. The residue from the $CHCl_3$ extract on chromatography over alumina afforded (±)N-acetylxylopine (7) crystallising from $CHCl_3$–MeOH in fine needles (10 mg), m.p. 214°, optically inactive, identified by direct comparison (m.m.p., co-TLC, UV and IR) with an authentic sample.

Chromium trioxide-pyridine oxidation of michelanugine (4) *to lanuginosine* (2). Michelanugine hydrochloride (50 mg) in pyridine (2 ml) was treated with CrO_3 (100 mg) in pyridine (2 ml) for 1 hr in the cold. EtOH (1 ml) followed by water (10 ml) was added and the soln was extracted thoroughly with $CHCl_3$. The combined $CHCl_3$ layer was extracted repeatedly with 5% HCl aq. The acid layer was basified and extracted with $CHCl_3$. The orange residue obtained by removal of solvent from the dried $CHCl_3$ extract contained some pyridine which was removed by azeotropic distillation with about 50 ml of C_6H_6 and finally in the vacuum, was chromatographed over alumina (2 g) and eluted with C_6H_6 and $CHCl_3$. The residue from the $CHCl_3$ eluate crystallised from $CHCl_3$ to give lanuginosine as orange needles (5 mg), m.p. 316–318°(d). The identity was established through direct comparison with natural lanuginosine (m.m.p., TLC, UV and IR).

Clemmensen reduction of michelanugine (4) *to xylopine* (6). Michelanugine hydrochloride (15 mg) in glacial HOAc (0·5 ml) was added to freshly prepared Zn–Hg (500 mg) immersed in a mixture of water (1·5 ml) and conc. HCl (3·5 ml). The mixture was heated on a water bath for 6 hr, 0·5 ml conc. HCl being added twice at intervals of 3 hr and then heated for further 3 hr. The supernate was decanted off and the remaining Zn–Hg was washed with water. The combined solution was cooled, strongly basified with NH_3 and extracted thoroughly with $CHCl_3$. The residue obtained by evaporation of the solvent from the washed and dried $CHCl_3$ extract showed similar TLC behaviour as that of xylopine. The residue also showed comparable absorption maxima in EtOH (237, 280, 319 nm) in qualitative UV as that of xylopine. The residue which could not be crystallised due to paucity of material and some accompanying impurities, was treated with Ac_2O (0·3 ml) and fused NaOAc (25 mg) and left overnight. The mixture was treated with water, neutralised with $NaHCO_3$ and extracted with $CHCl_3$. The residue from the dried $CHCl_3$ extract on chromatography over alumina afforded a slightly laevorotatory microcrystalline solid (~1 mg), m.p. 214°, from $CHCl_3$–MeOH, identified as N-acetylxylopine by direct comparison through m.m.p., qualitative UV (217 and 283 nm) and co-TLC with an authentic sample

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