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Investigations on *Litsea polyantha* Juss. Isolation and Identification of Actinodaphnine

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From the bark of *Litsea polyantha* Juss. β-sitosterol and actinodaphnine, an aporphine alkaloid, previously isolated from *Actinodaphne hookeri* Meissn, have been reported. The identity was established through UV, IR, NMR and Mass spectral evidences.

The bark of the plant *Litsea polyantha* Juss. (N. O. Lauraceae) is widely used in the indigenous system of medicine in India as astringent, stomachic, stimulant and also used in diarrhoea[1]. However, no systematic study was found to be carried out with this plant.

From the petrol ether extract of the bark after saponification and alumina chromatography of the non-saponified fraction, β-sitosterol was isolated at a fairly good yield. Two other minor constituents, m.p. 79-81° and 217-8° have been isolated from the chromatographic fractions and are being investigated.

Almost the total crude base could be extracted by chloroform at *p*H 4 from the ethanol extract of the defatted bark. Thin layer chromatogram as well as paper chromatography indicated the crude base to be a single compound. Further increase of the *p*H to 7-8 of the aqueous acid extract gave a small quantity of the same alkaloid.

The crude alkaloid was purified by crystallisation from chloroform and then from ethanol (95%). It was obtained in colourless prisms, $C_{18}H_{17}NO_4$(M^+=311); m.p. 204—5° $(\alpha)_D^{31°}$, + 59.8° (methanol); λ_{max}^{MeOH} 282 (logϵ, 4.42) and 307 m μ (logϵ, 4.18); $\lambda_{max}^{N/10}$ aq. NaOH, 322 mμ (logϵ, 4.42). The base gave a mono-hydrochloride, m.p. 282-83.5° (decomp.); $(\alpha)_D^{31°}$, +13.68° (water, having similar UV Spectrum as the base; an N, O-diacetyl derivative, m.p. 252-3°; $(\alpha)_D^{31°}$, +327° (methanol), λ_{max}^{MeOH} 268 (logϵ, 4.06), 276 (logϵ, 4.12) and 305 m μ (logϵ, 4.05) and a mono-picrate, m.p. 222-3°.

The physical constants of the hydrochloride and the picrate of the base corresponded to those of actinodaphnine, an aporphine alkaloid isolated from *Actinodaphne hookeri* Meissn. (N.O. Lauraceae)[2]. Repeated crystallisation of the base did change neither its melting point nor the optical rotation which were found to be somewhat different from those reported for actinodaphnine. As further physical data on actinodaphnine was lacking (Lit.[2,3]) it was considered worthwhile to establish the identity of the alkaloid as actinodaphnine.

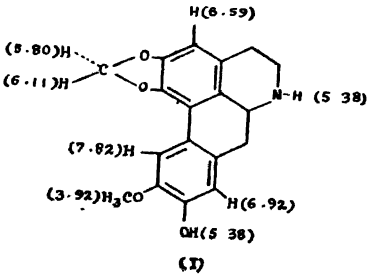
1. R. N. Chopra, S. L. Nayar and I. C. Chopra, Glossary of Indian Medicinal Plants (Council of Scientific & Industrial Research, New Delhi), 1956, p. 155.
2. S. Krishna & T. P. Ghose, *J. Ind. Chem. Soc.*, 1932, **9**, 429.
3. T. P. Ghose, S. Krishna and E. Schlittler, *Helv. Chim. Acta*, 1934, **17**, 919.

The UV absorption of the free base is similar to that of glaucine[4], a 1, 2, 9, 10-tetra-oxygenated aporphine and that for the diacetyl base is comparable with the spectrum of 1, 2, 10-trimethoxy aporphine, λmax 267 (logϵ, 4.14), 276 (logϵ, 4.40) and 304 mμ (log ϵ, 3.87)[5]. Obviously the alkaloid is tetra-oxygenated and it contains an acylable phenolic hydroxyl group.

An interesting observation is the strong hydrogen bonding of the hydroxyl group. It indicated a negative ferric chloride test and also the OH-stretching frequency was observed as a broad peak around 2740 cm^{-1} indicating a steric congestion forcing the hydroxyl group close to other functional group causing an association. Formation of hydrochloride of the base removed this hydrgoen bonding as manifested by positive ferric chloride colouration and appearance of a sharp peak at 3289 cm^{-1}. Similar observations for hydrogen bonding are quite familiar in cases of aporphine alkaloids[6]. Bands at 2551 cm^{-1} and 2500 cm^{-1} in the base, HCl indicated $>NH^{+}_{2}$, and 1773 cm^{-1} for Ph-OAC and 1661 cm^{-1} for N-AC in the diacetyl base confirms the free hydroxyl as well as free-NH groupings in the free base. Presence of methylenedioxy group in the base is also evident from the bands at 1049 cm^{-1} and 942 cm^{-1}.

The chemical shift of the methoxyl group in the proton nuclear magnetic resonance spectra (3.92δ) corresponds[7] to that at C-9 or C-10 whereas the presence of methylenedioxy group is evident from the two doublets centered at 5.80 and 6.11δ. The aromatic hydrogens are found at a downfield (6.59, 6.92 and 7.82δ) and the pattern is comparable with dicentrine[8].

The two protons centered at 5.38δ corresponds to the protons of -NH and -OH groups which disappears on deuteration as well as acetylation.



(I)

(I) (Parantheses indicate δ values)

The molecular ion peak in the mass spectrum of the base is found at m/e 311 (Fig. 1) and that of the diacetyl derivative at m/e 395 (Fig. 2) with a strong (M-1) peak (base peak) characteristic of aporphines in the spectrum of the base. In both the compounds the diagnostic peaks for aporphines at m/e 152 and 165 were observed[9]. The peaks at

4. J. Cohen, W. Von Langenthal and W. I. Taylor, *J. Org. Chem.*, 1961, **26,** 4143.
5. T. Kitamura, *J. Pharm. Soc. Japan.*, 1960, **80,** 1104.
6. A. R. Katritzky, R. A. Y. Jones and S. S. Bhatnagar, *J. Chem. Soc.*, 1960, 1950.
7. I. R. C. Bick, J. Harley-Mason, N. Sheppard and M. J. Vernango, *J. Chem. Soc.*, 1961, 1896.
8. N S. Bhacca, L. F. Johnson and J. N. Shoolery, "NMR Spectra Catalogue" (Varian Associates, Palo Alto, Calif.), 1962.
9. M. Shamma and W. A. Slusarchyk, *Chem. Rev.*, 1964, **64,** 59.

m/e 296 (M-15) and 279 [(M-1)-31] in the base corroborates the observation that the alkaloid is methoxylated. The genesis of the peak at m/e 279 is corroborated by the appearance of the metastable peak at m/e 251.1. Appearance of a peak at m/e 282 (M-29) due to expulsion of-CH_2=NH supports the presence of free NH group in the molecule.

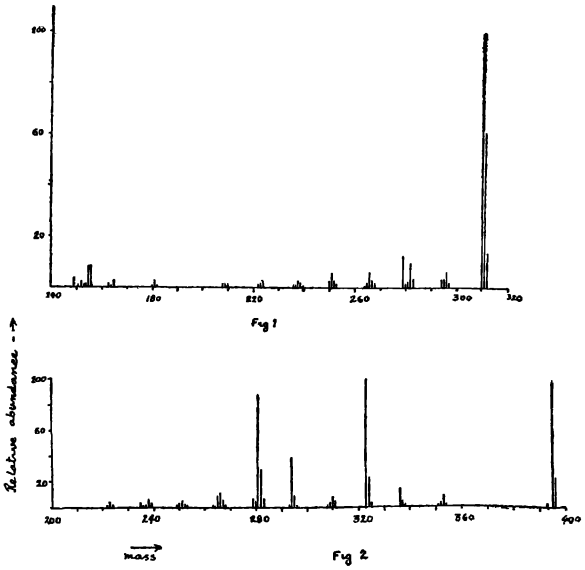
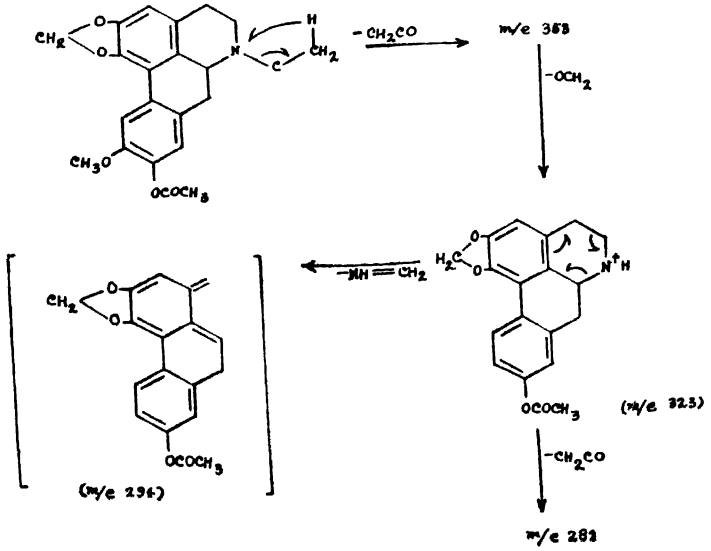


Fig 1

Fig 2

The mass spectra of the diacetyl base corroborates the conclusions drawn from the spectra of the base. In this case absence of (M-1) peak is due to the fact that before the molecular ion looses a proton (as in the case of base) immediate fragmentation of the N-acetyl group takes place with the elimination of ketene. The elimination of ketene as well as other fragmentation patterns of the diacetyl base are shown below :



The chemical and spectral evidences clearly show that the isolated alkaloid is identical with actinodaphnine, thus recording its isolation from a new source.

EXPERIMENTAL

All melting points are corrected. The IR spectra was taken in Nujol-mull. NMR spectra was taken in a Varian A-60 in $CDCl_3$ solution with TMS as internal reference.

Isolation of β-sitosterol and actinodaphnine : The finely ground bark (1 kg.) of *Litsea polyantha* was defatted with petroleum ether (60-80°) and the defatted marc after moistening with dilute ammonia was percolated with ethanol (95%) (4.5 l.) till the percolate showed negative test with Mayer's reagent. The concentrated percolate was brought to *p*H 4 with acetic acid. The whole solution was freed from tannins and other impurities by filtration and extracted exhaustively with chloroform (2 l.) The extract was concentrated to a small volume and kept in the cold when a good amount of crystal was obtained. Further concentration and cooling deposited a small amount of 2nd crop (total yield 0.5%). Repeated crystallisation first from chloroform and then from ethanol (95%) afforded colourless prisms; m.p. 204-5° (decomp.) $(\alpha)_D^{31°}$+59.8 (C, 1.1 in methanol). (Found: C,69·14 H, 5.56 ; N, 4.63 ; -OCH_3, 9.74. $C_{18}H_{17}NO_4$ requires C, 69.45; H, 5.50 ; N, 4.50; ; -OCH_3, 9.97).

(*a*) The base, HCl was prepared in the usual way. Crystallisation from ether-alcohol mixture gave white needles; m.p. 282-83.5° ; $(\alpha)_D^{31°}$+13.68° (C, 1.5 in water). (Found: C, 62.36 ; H, 5.34 ; N, 3.92 ; Cl, 9.96. $C_{18}H_{18}NO_4$ Cl requires C, 62.18; H, 5.19 ; N, 4.03 ; Cl, 10.22).

(*b*) The picrate was prepared in the usual way. Crystallisation from alcohol gave yellow needles ; m.p., 222-3°. (Found: C, 52.92 ; H, 3.61 ; N, 9.98. $C_{18}H_{17}NO_4$. $C_6H_3N_3O_7$ requires C, 53.33 ; H, 3.70 ; N, 10.37).

Acetylation of actinodaphnine: Actinodaphnine (0.2 g.) was kept overnight at room temperature with a mixture of acetic anhydride (2 ml.) and pyridine (1 ml.) After usual work up, the crude material was crystallised twice from ethanol (95%) in silky needles, m.p. 252.3° ; $(\alpha)_D^{31°}$+327° (C, 0.8 in methanol). (Found: C, 66.94; H, 5.25 ; N, 3.57. $C_{22}H_{21}NO_6$ requires C, 66.83 ; H, 5.32 ; N, 3.54).

Isolation of β-sitosterol : The dark brown gum (10 g.), obtained on removal of the solvent from the petrol ether extract, was saponified in the usual manner with ethanolic potassium hydroxide. The unsaponifiable matter (3 g.) was separated and subjected to chromatography over Brockmann alumina (100 g.) using light petroleum, benzene, ether, chloroform, alcohol and their mixtures as eluents. The petrol ether (40°-60°) : benzene (9 : 1) fraction afforded white flakes which after recrystallisation from petrol ether (40°-60°) gave colourless flakes, m.p. 79-80°. The fractions eluting with benzene: ether (7:3 and 9 : 1) afforded good amount of solid. This on crystallisation from methanol afforded flakes, m.p.; 137-8° ; $(\alpha)_D^{31°}$, -38° (C, 0.92 in chloroform). (Found : C, 84.1 ; H, 12.5. $C_{29}H_{50}O$ requires C, 83.99 ; H, 12.15) ; m.m.p. with authentic β-sitosterol, 137°. Thin layer chromatogram, R_f 0.42 [solvent : Petrol ether (40°-60°): chloroform : 1 : 5].

β-Sitosterol acetate : The above solid (0.1 g.) was acetylated with pyridine (1 ml.) and acetic anhydride (1 ml.) in the usual manner. The acetate on crystallisation from methyl alcohol gave β-sitosterol acetate (0.08 g.), m.p. 124-6°. (Found : C, 81.60 ; H, 11.22. $C_{31}H_{52}O_2$ requires C, 81.52 ; H, 11.48).

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