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ISOLATION AND STRUCTURE OF A NEW ALKALOID FROM THE ROOTS OF RAUWOLFIA SERPENTINA BENTH

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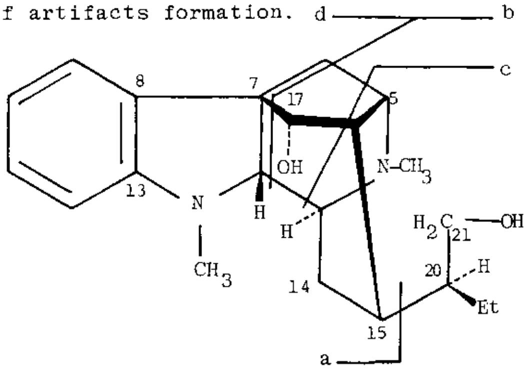
Abstract — A new dihydroindole alkaloid sandwicoline has been isolated from undried winter roots of Rauwolfia serpentina of Nepalies origin and its structure determined as 21-monohydro-N-methylsandwicine through chemical and spectral studies.

The present work was undertaken in the alkaloidal constituents of Nepalian roots of Rauwolfia serpentina in the light of earlier observations that the alkaloidal constituents show significant regional variations.[1-4] As a result, a new dihydroindole alkaloid, hitherto unreported from any of the various species of Rauwolfia,[5-8] has been isolated from the ethyl acetate soluble fraction of the aqueous extract and provisionally named as sandwicoline (I). In the course of its isolation care has been taken to employ the mildest possible procedure in order to exclude the possibility of artifacts formation.



(I)

Sandwicoline has molecular formula $C_{21}H_{30}N_2O_2$ (elemental analysis and high resolution mass M^+ 342.2307). The uv spectrum showed maxima at 250 and 295nm, characteristic of dihydroindole alkaloids. The ir spectrum showed O-H stretching at 3350 cm^{-1} in addition to other peaks at 2890-3100 cm^{-1} (C-H stretching) 1610, 1465 cm^{-1} (C=C vibration of the benzene ring), 775 and 880 cm^{-1} (C-H bending of substituted benzene ring). There is no strong peak between 1630 and 1800 cm^{-1} showing the absence of carbonyl group in the alkaloid.

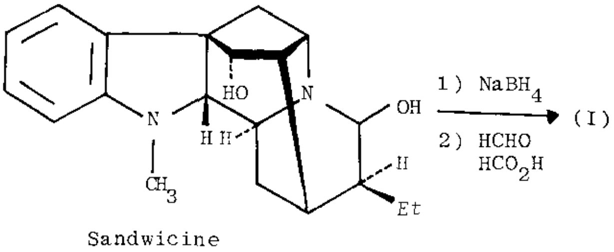
The calculation of ring equivalents and close similarity of the spectral data with sandwicine, provided substantial evidence of the structure of sandwicoline being closely related to that of sandwicine.[9] ^{1}H-nmr (see experimental) and ^{13}C-nmr (Table) data showed the presence of two N-methyl and two hydroxyl groups. Presence of the latter was further supported by the formation of its diacetyl derivative (II).

It does not reduce the ammonical silver nitrate solution showing the absence of carbinol amine-aldehyde imine tautomerism, indicating the opening of $C_{21}-N_4$ bond.

The mass spectrum showed, apart from the molecular ion, peaks at m/e 324.2201 $(M-H_2O)^+$, m/e 313.1916 $(M-C_2H_5)^+$ and m/e 311.2123 $(M-CH_2-OH)^+$. The fragment ions at m/e 269.1651 $(C_{17}H_{21}N_2O)^+$, m/e 198.1493 $(C_{11}H_{20}NO_2)^+$, m/e 168.1390 $(C_{10}H_{18}NO)^+$ and m/e 144.0813 $(C_{10}H_{10}N)^+$ correspond to the ions a,b,c and d respectively.

The ^{1}H-nmr spectrum showed a multiplet at δ 6.54-7.24 related to aromatic protons, a two-protons doublet at δ 3.66 (H-21, J=6Hz), two-three-protons singlets at δ 2.70 and δ 2.51 (N_1-CH_3 and N_4-CH_3 respectively), a triplet at δ 0.96 (H-19) and a multiplet at δ 1.25 (H-18). A one-proton doublet at δ 4.54 (J=9Hz) has been attributed to (H-17). In the ^{1}H-nmr spectrum of II the signals of H-17 and H-21 shifted to δ 4.70 and δ 5.34 respectively. The data recorded so far collectively led to the formulation of structure I for sandwicoline which was further supported by ^{13}C-nmr. Thus the nature of C-21 and presence of N_4-CH_3 was confirmed by the appearance of two signals at δ 79.9 and δ 28.7 respectively, and spin echo spectrum.

Regarding the stereochemistry of C-17, it has been conclusively shown to have the same configuration as that of sandwicine since H-17 exhibits a coupling constant of 9Hz which is comparable with that of sandwicine as against J≈1.8Hz observed in ajmaline.[10] Further, the identity of the stereochemistry of the base and sandwicine with respect to all asymmetric centres was corroborated through comparison of its various data (ir, uv, mass, nmr, mp and sp. rotn) with those of the compound obtained from sandwicine through its reduction ($NaBH_4$) followed by methylation (HCHO/HCOOH).



EXPERIMENTAL

Melting points were recorded in glass capillary tubes and are uncorrected. ir and uv spectra were measured on JASCO IRA-I spectrometer and Pye-Unicam SP-800 spectrometer respectively. Mass spectra were recorded on Finnigan MAT 112 and MAT 312 double focussing mass spectrometer connected to PDP 11/34 computer system. ^{1}H and ^{13}C nmr (Broad band and Gated spin echo) spectra were recorded in $CDCl_3$ on a Bruker WP-100 SY FT-NMR spectrometer with T.M.S. as internal reference. ^{13}C-nmr spectral assignments have been made partly through a comparison of the chemical shifts with the published data for similar compounds[11] and partly through the appearance of signals in the spin-echo spectrum. The purity of samples was checked on t.l.c. (silica gel SIF-254 precoated aluminium cards).

Isolation

The undried winter roots of Rauwolfia collected from Khatmando (Nepal) were chopped into small bits and repeatedly extracted out with water at room temperature. The combined extracts were freeze-dried and divided into ethyl acetate soluble and insoluble fractions. The ethyl acetate soluble fraction was shaken out with 2% acetic acid which was ammoniated to pH 7.5 and extracted out with ethyl acetate. The ethyl acetate solution was washed, dried over anhydrous sodium sulfate and freed of the solvent under reduced pressure. The residue, thus obtained was subjected to thick layer chromatography (silica gel, chloroform-methanol 9.5:0.5) resulting in the isolation of sandwicoline as white crystalline solid which on recrystallization from methanol formed elongated rods, mp 177-178°C, $|\alpha|_D^{20} = +202°$ ($CHCl_3$). EIMS m/e (rel. int.%) 342.2312 (M^+, calcd. for $C_{21}H_{30}N_2O_2$, 342.2307) (10), 269.1651 $(C_{17}H_{21}N_2O)^+$ (6), 198.1493 $(C_{11}H_{20}NO_2)^+$ (100), 168.1390 $(C_{10}H_{18}NO)^+$ (20) and 144.0813 $(C_{10}H_{10}N)^+$ (12). It analyzed for $C_{21}H_{30}N_2O_2$ (observed C=73.49, H=8.94, N=8.28, O=9.29%, calcd. for $C_{21}H_{30}N_2O_2$ C=73.68, H=8.77, N=8.19, O=9.37%); I.R. ν_{max}(cm^{-1}) ($CHCl_3$): 3350 (O-H stretching), 1375 (O-H bending) 3100, 1450 (aromatic vibration); U.V. λ_{max}(nm) (MeOH): 210, 250 and 295. ^{1}H-nmr ($CDCl_3$): δ 6.54-7.24 (4H, m, aromatic protons), 4.54 (1H, d, J = 9Hz, H-17), 3.66 (2H, d, J = 6Hz, H-21), 3.35 (2H, m, H-3 and H-5), 2.70 (3H, s, $N_1 - CH_3$), 2.51 (3H, s, N_4-CH_3), 0.96 (3H, t, H-18) and 1.25 (2H, m, H-19).

TABLE

^{13}C-NMR CHEMICAL SHIFTS

Carbon No.	Chemical shift	Carbon No.	Chemical shift
2	75.2	14	31.0
3	44.8	15	27.7
5	53.1	16	34.0
6	34.3	17	70.4
7	54.0	18	11.9
8	131.2	19	23.0
9	119.9	20	47.2
10	118.6	21	79.9
11	127.0	N_1-CH_3	34.4
12	109.4	N_4-CH_3	28.7
13	153.8		

All values are in (ppm) relative to TMS = 0

Acetylation of I to II

To a solution of I (25 mg) in pyridine (1 ml), acetic anhydride (1 ml) was added and the reaction mixture was kept overnight at room temperature. On usual work up chromatographically pure II was obtained as a powder, which melted at 203-204^{o}C. I.R. ν_{max}(cm^{-1}) ($CHCl_3$): 1730 and 1620. EIMS m/e (rel.int.%) 426.2520 (M^+ calcd. for $C_{25}H_{34}N_2O_4$, 426.25184) (30), ^{1}H-nmr ($CDCl_3$): δ 6.60-7.31 (4H, m, aromatic protons), 5.70 (1H, d, J = 9Hz, H-17), 5.34 (2H, d, J = 6Hz, H-21), 2.10 and 2.02 (3H each s, 2 x $OCOCH_3$) sandwicoline has also been characterised through following salts:

Sandwicoline Hydroiodide

Hydroiodide was prepared by adding potassium iodide to the solution of the base in dilute acetic acid. It formed rectangular plates from methanol which melted at 112-113^{o}C. It is insoluble in ether, petroleum ether and readily soluble in methanol, ethanol and acetone.

Sandwicoline Picrate

Picrate was obtained as a bright yellow microcrystalline powder by adding the aqueous solution of picric acid to the acetic acid solution of the base. It melted at 153-154^{o}C. It is sparingly soluble in ether, soluble in ethanol and methanol and readily soluble in acetone and water.

Dihydrosandwicine

To a solution of 0.5g of sandwicine in 10ml of methanol, 1.0g of sodium borohydride was gradually added with stirring. After keeping it at room temperature for 3 h, the excess of borohydride was destroyed by adding 10% acetic acid. The reaction mixture was then basified with 20% ammonium hydroxide and extracted out with ethyl acetate. On usual work up of the ethyl acetate layer, dihydrosandwicine was obtained as a crystalline residue (yield theoretical) which on recrystallization from acetone formed coarse hexagonal rods, mp 210-212°C; $|\alpha|_D^{20}$ + 102° ($CHCl_3$). EIMS m/z 328.21631 (41%, M^+) (calcd. for $C_{20}H_{28}N_2O_2$ 328.21506). It analyzed for $C_{20}H_{28}N_2O_2$ (observed C=72.89, H=8.48, N=8.60, O=10.03%, calcd. for $C_{20}H_{28}N_2O_2$C = 73.17, H=8.53, N=8.53, O=9.77%); I.R. $\nu_{max}(cm^{-1})$ ($CHCl_3$); 3345, 1370, 3100 and 1455; U.V. λ_{max}(n.m) (MeOH): 246 and 288.

21-Monohydro-N-methylsandwicine

Dihydrosandwicine (0.4g) was refluxed with 2.4 moles each of 40% aqueous formic acid and formaldehyde (40% solution) for 2 h. on the boiling water bath. The reaction mixture was then basified with ammonia and the liberated base extracted out with ethyl acetate, well washed with water, dried and freed of the solvent. 21-Monohydro-N-methylsandwicine obtained as a whitish residue crystallized from methanol and on recrystallization from the same solvent formed elongated rods. Its physical data were identical in all respects with those of sandwicoline.

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