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SEPARATION AND CHARACTERIZATION OF THE ALKALOIDS OF *SARCOCOCCA PRUNIFORMIS*

J. M. KOHLI, A. ZAMAN and A. R. KIDWAI

Department of Research in Unani Medicine, Tibbiya College and Department of Chemistry, Aligarh Muslim University, Aligarh, India

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Abstract—Two new steroidal alkaloids (A and B) isolated from *Sarcococca pruniformis* have been identified as 3α-dimethylamino-20α-methylacetylamino-5α-pregnane (I) and 3α-dimethylamino-20-methylacetyl amino-pregn-5-ene (II).

OCCURRENCE of an alkaloid "Saracocine" m.p. 236–238° in the Himalayan shrub *Sarcococca pruniformis* was first reported[1] and subsequently shown[2] to have the molecular formula $C_{26}H_{44}N_2O$ and it was suggested that the alkaloid was 3β-dimethylamino-20β-methylacetylamino-pregn-5-ene. In a later investigation,[3] this alkaloid was reported to have the structure 3β-dimethylamino-20α-methylacetyl-amino-pregn-5-ene.

In the present reinvestigation of the alkaloidal fraction from this plant, the petrol as well as alcohol extracts were found to contain several alkaloids as shown by the presence of a number of Dragendorff positive zones on paper chromatograms of the crude mixture. Repeated chromatography on alumina and fractional crystallization afforded the following crystalline bases: alkaloid A, m.p. 245–246°; B, m.p. 232–233°; C, m.p. 151–153° and a non-crystalline but paper chromatographically pure base D, m.p. 180–186°. The major portion of the crude base could not be obtained in a crystalline form and was found to be a mixture of several components. The two alkaloids, designated here as A and B, had very similar physical characteristics and could not be separated by simple chromatography over alumina using various solvent mixtures, or by fractional crystallization. Crystallization from methanol gave sharp melting colourless crystals which on chromatography over formamide impregnated paper were found to be mixture of two components. A better separation of the two constituents was obtained when the LAH reduction product of the mixture was run on phosphate buffer impregnated paper. The m.p. of the mixture varied between 240° and 246°.

A separation of the two bases in quantities sufficient for degradation was finally effected by repeated gradient elution chromatography over alumina using benzene–ether or benzene–ethyl acetate mixture for elution. The major component of this mixture is the higher melting alkaloid A which analysed for $C_{26}H_{46}N_2O$ and had a mol wt of 402 (mass spectrum) as required by the formula. There was no indication of any end absorption in its UV spectrum and it was shown by degradation to have

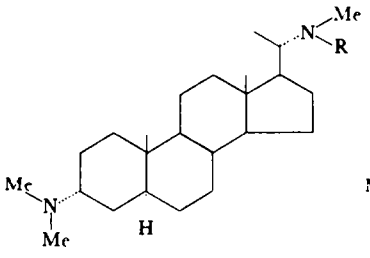
[1] I. C. Chopra and K. L. Handa, *Indian J. Pharm.* **13**, 129 (1951).

[2] K. L. Handa and O. E. Edwards, *IUPAC Symposium on the Chemistry of Natural Products*. Kyoto, April 12 (1964).

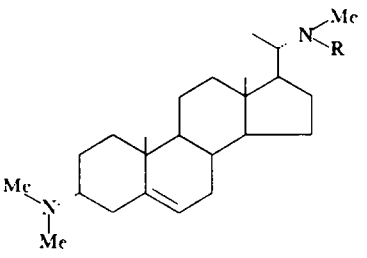
[3] A. Chatterjee, B. Das, C. P. Dutta and K. S. Mukherjee, *Tetrahedron Letters* No. 1, 67 (1965).

structure I with unknown stereochemistry at C_3 and C_{20} whereas alkaloid B was tentatively given structure II, as reported earlier.[4]

Structures I and II have now been confirmed for both these alkaloids and their stereochemistry have been established by direct comparison with known compounds.

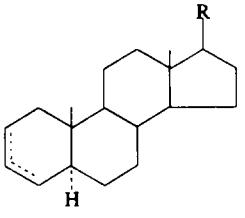


I: R = —AC
Ia: R = —Et
Id: R = H
Ie: R = —Me

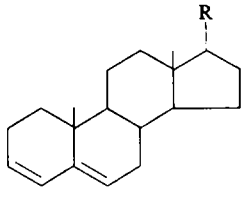


II: R = —AC
IIa: R = —Et
IId: R = H
IIe: R = —Me

Reduction of alkaloid A with LAH gave Ia, m.p. 151–153°. The reduction product when subjected to Hofmann degradation gave a mixture of olefines Ib which still showed the presence of nitrogen. Repetition of the Hofmann degradation to obtain a nitrogen free compound did not succeed and the olefinic mixture was recovered. This appears also to be the case with alkaloids of *Pachysandra terminalis* where too a nitrogen free compound could not be obtained on attempted Hofmann degradation of pachysandrine A.[5] The methochloride of the above mixture, however, undergoes Emde's degradation readily to give mixture of nitrogen free product Ic m.p. 78–81° in good yield.



Ib: R = —CH(Me)—NMe_2
Ic: R = —Et



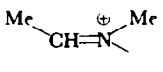
IIb: R = —CH(Me)—NMe_2
IIc: R = —Et

In the absence of a double bond at C_5 the Hofmann elimination from C_3 should give rise to the two double bond isomers pregn-2-ene and pregn-3-ene and, therefore, the nitrogen free product Ic was presumed to be a mixture of these two hydrocarbons. A separation in such cases is extremely tedious and was not attempted, instead the mixture was hydrogenated to a product which gave no depression in m.p. with 5α-pregnane prepared synthetically.

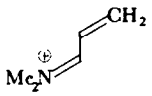
[4] J. M. Kohli, A. Zaman and A. R. Kidwai, *Tetrahedron Letters* No. 45, 3309 (1964).
[5] M. Tomita, S. Uyeo, Jr., and T. Kikuchi, *Tetrahedron Letters* No. 18, 1053 (1964).

The analysis of alkaloid B agreed with the formula $C_{26}H_{44}N_2O$, and hydrogenation indicated the presence of a single double bond. A similar sequence of reactions as with the alkaloid A, afforded a nitrogen free hydrocarbon IIc m.p. 85–86° which gave a red colour with 90% trichloracetic acid[6] and had the characteristic UV absorption of steroidal 3,5-dienes. On comparison with authentic pregn-3,5-diene[7] the two were found to be identical.

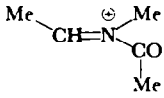
The mass spectrum of alkaloid A gave the following principal ion peaks *m/e* 58, 84, 100 and 110 characteristic of C_3, C_{20}-aminopregnane derivatives,[8,9] and associated with the generation of fragments (a), (b), (c) and (d) respectively.



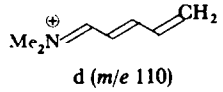
a (*m/e* 58)



b (*m/e* 84)



c (*m/e* 100)



d (*m/e* 110)

The NMR spectrum of alkaloid A is complicated due to the restricted rotation of the 17β—CH(Ac)—(N—Me)Me grouping and is comparable to that of epipachysamine-A.[10] Signals for amide N—Me occur at 7·28, 7·33 (2 peaks); for the N (Me_2) at 7·83, for —Ac at 7·96, 8·04 (2 peaks). The signals for secondary C—Me occur at 8·72, 8·83, 8·93 (3 peaks) and for the tertiary C—Me at 9·24, 9·33 τ (2 peaks).

The mass spectrum of alkaloid B gave the following principal ion peaks *m/e* 84, 100 and 58. The absence of ion peak *m/e* 110 is in agreement with the presence of a double bond at C_5. The NMR spectrum of alkaloid B gave the following signals 4·77 (Multiplet, olefinic proton), 7·28, 7·32 (2 peaks; amide N—Me), 7·85 N(Me_2), 7·96, 8·04 (2 peaks; Ac), 8·78, 8·81, 8·92 (3 peaks; sec Me) and 9·02, 9·27 τ (3 peaks; te-Me). The spectrum was thus similar to that of alkaloid A except for the presence of a signal for an olefinic proton and a down field shift of the signal of the C_{10} tertiary Me. Here also the secondary C—Me gave a triplet attributed earlier to restricted rotation of the 17β—CH(Ac)(N—Me)Me grouping.

Determination of the configuration at C_3 and C_{20} by comparison with compounds of known stereochemistry required preparation of the desacyl derivatives of the two bases. Attempts to cleave the Ac groups of the two bases by treatment with acids or

[6] R. D. Haworth, J. McKenna and G.H. Whitfield, *J. Chem. Soc.* 3127 (1949).

[7] R. D. Haworth, L.H. C. Lunts and J. McKenna, *J. Chem. Soc.* 3749 (1956).

[8] W. Vetter, P. Longevialle, F. Khuong-Huu-Laine (Mme) Q. Khuong-Huu et R. Goutarel, *Bull. Soc. Chim.* 1324 (1963).

[9] H. Budzikiewixz, *Tetrahedron* **20**, 2267 (1964).

[10] T. Kikuchi, S. Uyeo, Jr., M. Ando and A. Yamamoto, *Tetrahedron Letters* No. 27, 1817 (1964).

alkalies did not succeed. Kikuchi and *et al.*[10] used phenyl–lithium in benzene–ether to cleave the Ac group of epipachysamine-A, but this method also failed to give a crystalline product in the present case. The procedure employed by Goutarel for the cleavage of the Ac group from similar alkaloids—and very kindly made available to us in a personal communication was therefore adopted. This consisted of treating the Ac compound with lithium in ethylamine solution at 0°. The desacyl compounds Id and IId of the two bases were obtained in fairly satisfactory yields. Methylation of the desacyl compounds Id and IId obtained from alkaloids A and B afforded the corresponding bisdimethylamino compounds Ie and IIe which on comparison with 3α,20α-bisdimethylamino-5α-pregnane[11] and 3α,20α-bisdimethylamino-pregn-5-ene[12] were found to be identical by mixed m.p. and IR spectra (Fig. 1 and Fig. 2) respectively.

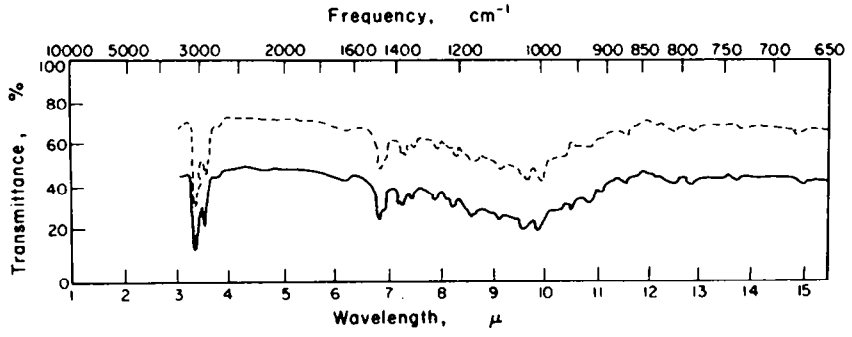


FIG. 1.

..... 3α-20α-Bisdimethylamino-5α-pregnane, from alkaloid.
——— 3α-20α-Bisdimethylamino-5α-pregnane, authentic.

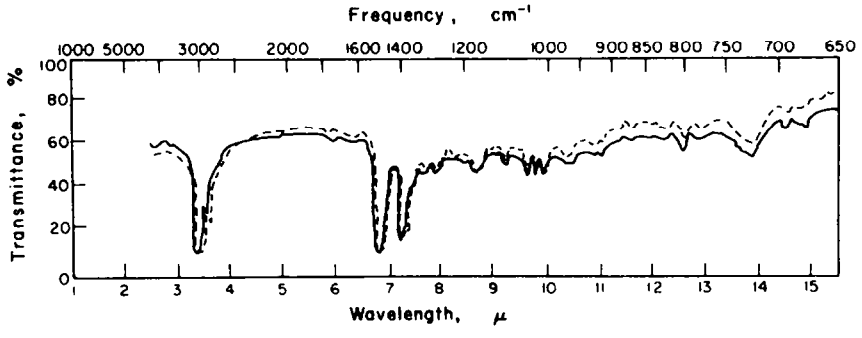


FIG. 2.

..... 3d-20α-Bisdimethylamino-pregn-5-ene, from alkaloid.
——— 3d-20α-Bisdimethylamino-pregn-5-ene, authentic.

[11] M. M. Janot, Q. Khuong-Huu, F. Laine et R. Goutarel, *Bull. Soc. Chim.* 111 (1962).
[12] V. Cerny, L. Labler et F. Sorm. *Chem. Listy* **51**, 1351 (1957).

EXPERIMENTAL

All UV spectra were measured on a Beckmann model DU instrument in 95% EtOH. IR spectra were taken on a Perkin Elmer Spectrometer model No. 21 either in Chf soln, as KBr pellets or mulls in Nujol. NMR spectra were measured in $CDCl_3$ and chemical shifts are reported in τ values, using TMS as the internal reference.

Air dried leaves of *Sarcococca pruniformis* (5 Kg) were extracted with pet. ether (60–80°) by percolation at room temp. The gummy green residue obtained on removal of the solvent was redissolved in a minimum amount of pet. ether and the soln kept overnight in ice chest, filtered from the deposited solid (Betulin) and extracted with dil HCl aq. The aqueous acidic layer was extracted repeatedly with ether, basified with ammonia and the precipitated alkaloid extracted with Chf. The process was repeated 3 times to give crude alkaloid (1 g). This mixture was chromatographed over alumina (100 g, E. Merck). The combined benzene–petrol eluate on evaporation gave a solid (150 mg), which was crystallized from benzene–pet. ether. The first crop of crystals was shown by paper chromatography to be a mixture of alkaloids A and B only. The second crop obtained on concentration of the mother liquor contained a third minor alkaloid C along with A and B. The minor alkaloid C was separated from alkaloids A and B by repeated chromatography over alumina and crystallized from benzene–pet. ether, m.p. 151–153° (6–7 mg). The petrol exhausted leaves were percolated with EtOH at room temp and the combined extracts from 4 such percolations were evaporated to dryness under vacuum. The gummy residue was dissolved in Chf and extracted with dil HCl aq. The aqueous layer was washed several times with ether and then basified with ammonia. The precipitated alkaloid was again taken in Chf and the above process repeated 3 to 4 times. Evaporation of the solvent gave crude alkaloid (3·5 g), which was chromatographed over alumina (150 g) using benzene and benzene–acetone (9:1) for elution.

Paper chromatograms of benzene and benzene–acetone fractions showed 3 Dragendorff positive spots. On comparison two of the spots could be assigned to alkaloids A and B of the pet. ether extract, whereas the third alkaloid D was present only in traces and was easily separated by a second chromatography. Alkaloids still retained on the column were washed down with MeOH. Evaporation of MeOH gave a multicomponent mixture (2 g). The mixture of alkaloids A and B from petrol as well as from the EtOH extract was chromatographed over alumina using either benzene–benzene or benzene–AcOEt mixtures for gradient elution. 50 fractions of (10 ml) each were collected (cf. Tables 1 and 2). The purity of the fractions was checked by paper chromatography. The mixed fractions were rechromatographed to give a further quantity of alkaloids A and B. In this way alkaloid A (120 mg) and alkaloid B (30 mg) were separated.

TABLE 1.

No. of Fractions	Percentage of ether in the eluate	Nature of the alkaloid
1 to 20	44 to 48%	Alkaloid A
20 to 35	48 to 51%	Mixture of alkaloids A and B
35 to 50	51 to 55%	Alkaloid B

TABLE 2.

No. of Fractions	Percentage of ethyl acetate in the eluate	Nature of the alkaloid
1 to 13	32 to 35%	Alkaloid A
13 to 38	35 to 40%	Mixture of alkaloids A and B
39 to 50	40 to 43%	Alkaloid B

Alkaloid A. m.p. 245–246° (MeOH), $(\alpha)_D^{23} = -14°$ (Chf). (Found: C, 77·26; H, 11·33; N, 7·33; $C_{26}H_{46}N_2O$ requires: C, 77·55; H, 11·52; N, 6·96%).

Alkaloid B. m.p. 232–233° (MeOH), $(\alpha)_D^{23} = -56·4°$ (Chf). (Found; C, 77·8; H, 11·1; N, 6·8; $C_{26}H_{44}N_2O$ requires: C, 77·94; H, 11·07; N, 6·99%).

Methiodide of alkaloids A and B. Alkaloid A (500 mg) in Chf (6 ml) was refluxed with MeI (5 ml) for 3 hr. The residue obtained on evaporation of the solvent was crystallized from MeOH to give methiodide (650 mg) m.p. 273–274°.

The methiodide of alkaloid B (150 mg) was prepared as above and had m.p. 256–258°.

Reduction of alkaloids A and B. A soln of alkaloid A (500 mg) in THF (30 ml) was added dropwise with stirring to a suspension of LAH (1 g) in ether (150 ml). The mixture was stirred at room temp for 4 hr, left overnight and finally refluxed for 1 hr. The resulting complex was decomposed by the slow addition of water, the ether layer was separated and worked up to give Ia (400 mg), m.p. 151–153° $(\alpha)_D^{20} = +19·5°$ (Chf). (Found: C, 79·95; H, 12·09; N, 7·61; $C_{26}H_{48}N_2$ requires: C, 80·34; H, 12·45; N, 7·21%).

The reduction of alkaloid B (500 mg) was carried out as above, which afforded IIa (400 mg) as colourless needless from MeOH m.p. 143–145°, $(\alpha)_D^{27} = -39°$ (Chf). (Found: C, 80·50; H, 11·80; N, 7·15; $C_{26}H_{46}N_2$ requires: C, 80·76; H, 11·99; N, 7·25%).

Hofmann degradation of Ia *and* IIa. The product Ia (300 mg) in Chf (5 ml) was refluxed with MeI (4 ml) for 1 hr. The reaction mixture was kept overnight, diluted with ether and filtered to give the methiodide of Ia (375 mg). The methiodide (800 mg) was suspended in water (20 ml) and a suspension of Ag_2O in an equal amount of water was added. The mixture was shaken for 4 hr, filtered, the residue washed thoroughly with water and the combined filtrate evaporated to dryness in vacuum. The dried material was decomposed by heating in an oil bath for 30 min, at 200° in vacuum, the product taken in benzene and extracted with dil HCl. Basification of the aqueous layer and extraction with ether afforded a brownish product which was purified by chromatography over alumina using benzene for elution to give a mixture of double bond isomers Ib (275 mg). Crystallization from acetone furnished colourless needless m.p. 104–107°. (Found: C, 83·56; H, 11·90; N, 4·60; $C_{23}H_{39}N$ requires: C, 83·82; H, 11·93; N, 4·25%).

Hofmann degradation of IIa (400 mg) was carried out under identical experimental conditions and the product IIb (125 mg) after crystallization from acetone had m.p. 107–108°, $(\alpha)_D^{27} = -130°$ (Chf). (Found: C, 84·47; H, 11·43; N, 4·25; $C_{23}H_{37}N$ requires: C, 84·34; H, 11·39; N, 4·28%).

Emde's degradation of Ib *and* IIb. The methiodide (300 mg) of Ib prepared as described earlier was refluxed with AgCl (400 mg) in MeOH for 4 hr. The reaction mixture was filtered and the filtrate evaporated to dryness *in vacuo* to give methochloride (250 mg). The methochloride (250 mg) was taken in water and treated with freshly prepared Na-Hg (5%) at 40–50° on a water bath for 4 hr. After keeping overnight, the aqueous soln was decanted and extracted with ether. Evaporation of ether gave Ic (120 mg) which crystallized from acetone as colourless needless m.p. 78–81°. (Found: C, 87·92; H, 11·58; $C_{21}H_{34}$ requires: C, 88·04; H, 11·96%). Ic (100 mg) in glacial AcOH (10 ml) was hydrogenated over Adam's catalyst (25 mg) at atm press. After 5 hr the catalyst was filtered off and the soln diluted with distilled water. The separated product was filtered and crystallized to give colourless needless m.p. 83–84° (70 mg). (A mixed m.p. with authentic 5α-pregnane was 83–84°.

Emde's degradation of IIb (200 mg) was carried out as above and the hydrocarbon IIc (60 mg) was crystallized from acetone m.p. 85–86°. (Found: C, 88·42; H, 11·52; calc. for $C_{21}H_{32}$: C, 88·66; H, 11·34%).

Deacetylation of alkaloid A. Alkaloid A (200 mg) was dissolved in $EtNH_2$ (20 ml), Li (300 mg) added in small portions with constant stirring to give an intense blue soln. The stirring was continued for another 15 min, followed by the addition of MeOH to destroy the excess reagent. The reaction mixture was diluted with water, extracted with CH_2Cl_2 and the resulting product Id (70 mg) was purified by chromatography over alumina using benzene and benzene–AcOEt (1:1) for elution. After crystallization from acetone it had m.p. 125–132°, $(\alpha)_D^{28} = +14·5°$ (Chf).

Methylation of Id. A mixture of Id (100 mg), formic acid (4 ml, 98%) and formaldehyde (6 ml, 40%) was refluxed for 8 hr, diluted with HCl (3 ml, 10%) and extracted with ether. The aqueous soln was basified with ammonia and worked up to give Ie (70 mg), which on crystallization from acetone had m.p. 164–165°. $(\alpha)_D^{20} = +16·5°$ (Chf). A mixed m.p. with authentic 3α, 20α-bisdimethylamino-5-αpregnane was 164–165°. (Found: C, 80·20; H, 12·50; N, 7·68; calc. for $C_{25}H_{46}N$: C, 80·15; H, 12·38; N, 7·48%).

Deacetylation and methylation of alkaloid B. Alkaloid B (100 mg) was deacetylated under the identical conditions and the product IId (≈40 mg) was methylated to give IIe which on crystallization from acetone had m.p. 148–149°, $(\alpha)_D^{28} = -35°$ (Chf). (Found: C, 79·63; H, 11·81; N, 7·34; calc. for $C_{25}H_{44}N_2$: C, 80·58; H, 11·90; N, 7·52%). A mixed m.p. with authentic 3α, 20α-bisdimethylamino-pregn-5-ene was 147–148°.

Synthesis of 5α-pregnane and pregna-3,5-diene. 3β-hydroxy-5α-pregnane (500 mg) prepared from pregnenolone was kept in pyridine (1·5 ml) with toluene-*p*-sulphonylchloride (500 mg) at 5° for 25 hr and then at 15° for 5 hr and worked up to give the toluene sulphonate m.p. 106–108° (500 mg). The above sulphonate (300 mg) on refluxing with dimethylaniline (2 ml) for 20 min gave a mixture of 5α-pregn-2 and 3-enes (220 mg), which on hydrogenation over Adam's catalyst gave 5α-pregnane m.p. 83–84° (200 mg).

Tosylation and detosylation of 3β-hydroxy-pregn-5-ene gave pregna-3,5-diene m.p. 85–87°.

Acknowledgement—The authors wish to thank Prof. F. Korte and Dr. H. Weitkamp for Mass spectra, Prof. R. Goutarel and Prof. F. Sorm for the samples of 3α, 20α-bisdimethylamino-5α-pregnane and 3α, 20α-bisdimethylamino-pregn-5-ene, Mr. N. K. Sharma for optical rotations and Council of Scientific and Industrial Research, New Delhi, for a fellowship to one of the authors (J.M.K.).