A NEW ASPIDOSPERMA ALKALOID: O-DEMETHYLASPIDOCARPINE[1]

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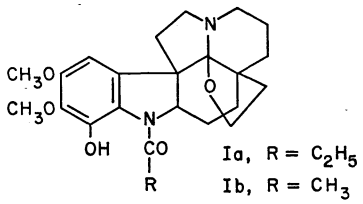
ABSTRACT

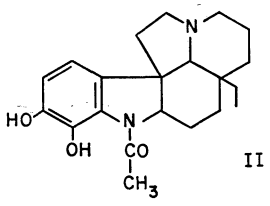
From the stem bark of *Aspidosperma album* four alkaloids have been isolated. Two of these (aspidoalbine and its N-acetyl-N-depropionyl homologue) have been reported recently and their structures established by Djerassi and his co-workers. The other two consist of aspidocarpine, and a new alkaloid, the structure of which has been determined. It is O-demethylaspidocarpine.

The alkaloids of the stem bark of *Aspidosperma album* (Vahl) R. Dent., collected in British Guiana,[3] have been under investigation in this laboratory for some time. A recent publication (1) reporting the structures of two alkaloids occurring in this plant prompts us to place on record some of our results.

Four bases have been isolated from the bark. Of these, alkaloids I and II are identical with aspidoalbine I*a* and its N-acetyl-N-depropionyl homologue I*b*, whose structures have



Ia, R = C_2H_5
Ib, R = CH_3



II

been determined by Djerassi[4] and his co-workers (1). Since they did not succeed in effecting a clean separation of these two bases and did not report the physical properties of the lower homologue, we now describe a method of separation and record the physical properties of both alkaloids (cf. Experimental). Thin layer chromatography tests showed that the method of separation was effective and produced both alkaloids in a chromatographically pure state.

Alkaloid I and its O-methyl derivative were compared directly with samples of aspidoalbine and O-methylaspidoalbine kindly supplied by Dr. C. Djerassi and found to be respectively identical. Alkaloid II analyzed correctly for N-depropionyl-N-acetylaspidoalbine. Both n.m.r. and infrared spectra indicated the presence of a hydrogen-bonded N-acetyl carbonyl. Alkaloid II gave rise to an O-methyl derivative which, although it had an infrared spectrum superimposable on that of N-depropionyl-N-acetyl-O-methylaspidoalbine, did not yield satisfactory analytical figures. The methyl derivative was hydrolyzed with hydrochloric acid and the product treated with propionic anhydride and pyridine. The purified product of the reaction showed no depression in melting point when mixed with O-methylaspidoalbine, and the two substances had the same optical rotation

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[3]*The plant material was obtained through the courtesy of Mr. R. Smeathers, Conservator of Forests, Kingston, Georgetown, British Guiana, whom we thank most cordially.*
[4]*We wish to thank Dr. C. Djerassi and Dr. B. Gilbert for their courtesy in giving us an advance copy of their manuscript.*

and superimposable infrared spectra. Hence alkaloid II is N-depropionyl-N-acetyl-aspidoalbine.

Alkaloid III appeared to be a new base not previously described while the fourth alkaloid proved to be identical in every respect with aspidocarpine (2). Alkaloid III was obtained from the ether eluate of a chromatogram of the crude alkaloids on a kieselguhr/phosphate buffer column. Its analytical figures corresponded to the empirical formula $C_{21}H_{28}O_3N_2$ (m.p. 156–158°). It was soluble in aqueous sodium hydroxide but not in sodium carbonate, and gave a violet coloration with a ferric chloride solution, so that at least one of its three oxygens must be present in a phenolic hydroxyl. It formed a monohydrochloride, m.p. 283°, and hence of the two nitrogens in the alkaloid one only appeared to be basic.

The n.m.r. spectrum of alkaloid III shows a striking resemblance to that of pyrifoline (3) and of aspidocarpine (2) except for the signals corresponding to the two methoxyl groups of the former and the one methoxyl of the latter, which are absent. The peak ($\delta = 11.0$) corresponding to the hydrogen-bonded hydroxyl in the n.m.r. spectrum of aspidocarpine (2) is also found in that of alkaloid III ($\delta = 11.3$). The ultraviolet spectrum (λ_{max} 227 mμ, log ϵ 4.25; 264 mμ, log ϵ 3.85) was similar to that of aspidocarpine (λ_{max} 228 mμ, log ϵ 4.42; 263.5 mμ, log ϵ 3.92). The infrared absorption spectrum contained bands indicative of hydroxyl groups and of an amide carbonyl. Since alkaloid III contained no methoxyl and its empirical formula contained CH_2 less than that of aspidocarpine it was thought probable in view of the foregoing spectral similarities that it could be O-demethylaspidocarpine. If alkaloid III be assumed to have the same skeleton as aspidocarpine, then a phenolic hydroxyl must be attached at C-17 because of its strongly hydrogen-bonded nature, evidenced in the infrared by the marked displacement to lower frequencies (1635 cm^{-1}) of the amide carbonyl absorption, and a characteristic signal in the n.m.r. spectrum.

The correctness of the assumption was confirmed by treatment of alkaloid III with dimethyl sulphate and sodium hydroxide. The reaction gave rise to a dimethyl derivative identical with O-methylaspidocarpine. Consequently, alkaloid III is O-demethylaspidocarpine and its structure is represented by formula II.

O-Demethylaspidocarpine provides a further example of a catechol type of alkaloid of which the first one reported in the aspidospermine series is spegazzinidine (5).

EXPERIMENTAL

All the n.m.r. spectra were taken with a Varian Associates A-60 instrument, and the infrared spectra with a Perkin–Elmer double-beam spectrophotometer Model 21B.

The ground stem bark was extracted first with petroleum ether and then with ethanol–ammonia (11 l. – 1 l.). A crystalline colorless material separated from the petroleum ether extract. It was heated with *n*-heptane to remove the accompanying fat. The insoluble crystalline basic substance was recrystallized from methanol, from which it separated as yellowish prisms. Several recrystallizations raised the melting point to 172–174°. A quantity of the same crystalline substance was also obtained from the basic ethanol extract. When the crude alkaloid of this extract was chromatographed in benzene solution on neutral alumina, the material was found in the first fractions of the eluate.

Aspidoalbine and N-Depropionyl-N-acetylaspidoalbine

The crystalline material was chromatographed on paper under the condition described by Taylor *et al.* (4) and found not to be homogeneous but to consist of two distinct components. This technique was modified to adapt it to the separation of relatively large amounts of material. Whatman No. 3 paper was used and the alkaloid mixture in chloroform was streaked along the base line. It was found convenient to use 240 mg of material for 130 cm of base line. When the chromatogram had developed and the paper had been allowed to hang overnight in air, thin vertical strips were cut from it at 15-cm intervals and sprayed with iodoplatinate reagent. This illustrated how the components had separated. The appropriate zones were cut out from the paper and each was thoroughly extracted with chloroform in a Soxhlet apparatus. Each extract

was evaporated to a volume of ca. 50 ml, was washed with 5% aqueous sodium carbonate and with water; it was dried over sodium sulphate and was evaporated to dryness. Each residue was dissolved in benzene, passed through a short column of alumina, and eluted with benzene; the products were crystallized from benzene–hexane and then from methanol. From 240-mg of mixture there was obtained 58 mg of alkaloid I (leading zone), colorless prisms, m.p. 174–177°, and 36 mg of alkaloid II (second zone), colorless prisms, m.p. 194–195°.

An easier but not complete separation of the two alkaloids can be achieved by chromatography of the mixture on low-activity (grade V) neutral alumina, and the use of *n*-hexane – chloroform as eluant. Under such conditions pure alkaloid I is first obtained, then a mixture of alkaloids I and II, and finally pure alkaloid II, but most of the mixture is separated into its components.

Alkaloid I, $[\alpha]_D^{25}$ +164° (*c*, 2.12 in chloroform). Found: C, 67.68; H, 7.71; N, 6.70; OCH_3, 14.68. Calc. for $C_{24}H_{32}O_5N_2$: C, 67.27; H, 7.53; N, 6.54; 2 OCH_3, 14.48%. A mixture of alkaloid I with a sample of aspidoalbine[5] (m.p. 168–173°) melted at 166–170°. On a silica gel chromatoplate with chloroform – *n*-hexane – diethylamine (10:20:3) as solvent, alkaloid I gave one spot while the sample of aspidoalbine gave two, one of which had the same mobility as the one spot of the former, and the other corresponded to alkaloid II.

On methylation with dimethyl sulphate and sodium hydroxide, alkaloid I gave rise to an O-methyl derivative, m.p. 128–131°, $[\alpha]_D^{25}$ +9.2° (*c*, 1.84 in methanol). Found: C, 68.17; H, 7.79; N, 6.30; OCH_3, 20.19. Calc. for $C_{25}H_{34}O_5N_2$: C, 67.85; H, 7.75; N, 6.33; 3 OCH_3, 21.04%. In admixture with a sample of O-methylaspidoalbine[5] (m.p. 120–123°), m.p. 120–132°. O-methyl alkaloid I gave one spot on a chromatoplate and the sample of O-methylaspidoalbine gave two, the major one of which corresponded in mobility with the one spot of the former.

Alkaloid II, m.p. 194–195°, had $[\alpha]_D$ +174° (*c*, 1.93 in chloroform). Found: C, 66.38; H, 7.29; N, 6.52; OCH_3, 15.06. Calc. for $C_{23}H_{30}O_5N_2$: C, 66.64; H, 7.30; N, 6.76; 2 OCH_3, 14.96%. λ_{max}^{EtOH} 227 mμ, log ε 4.43; 267 mμ, log ε 4.11. The only difference between the n.m.r. spectrum of alkaloid II and that of aspidoalbine is the replacement of the triplet at 1.26 δ (CH_3) and the quartet at 2.53 δ (CH_2) corresponding to the protons of the N-propionyl group of aspidoalbine by a peak at 2.36 δ corresponding to three protons (N-acetyl). The infrared absorption spectrum contains a band at 1625 cm^{-1} indicative of a hydrogen-bonded N-acetyl carbonyl. Alkaloid II therefore corresponds to the lower homologue of aspidoalbine.

Methylation of Alkaloid II

A quantity of alkaloid II was methylated with dimethyl sulphate and sodium hydroxide following the usual procedure. The product of reaction, after crystallization from aqueous methanol, was shown by thin layer chromatography not to be homogeneous. It was therefore chromatographed on a silica gel plate (20×20 cm), the main band (detected by spraying with water) scraped off and eluted with methanol–chloroform (1:1). The material recovered from the eluate was sublimed at 145° at 10^{-4} mm. Colorless needles were obtained, m.p. 169–173°, which after one more sublimation melted at 175–178.5°. This substance was found by thin layer chromatography to be homogeneous, and to have an infrared spectrum superimposable on that of N-depropionyl-N-acetyl-O-methylaspidoalbine.[5] The product, however, gave consistently low analytical figures. It was therefore hydrolyzed by boiling in 3 *N* hydrochloric acid under reflux for 2.5 hours. The solution was made alkaline and extracted with chloroform. The residue left after evaporation of the chloroform extract was heated on the steam bath for 2 hours with propionic anhydride and pyridine and the product of the reaction chromatographed on a silica gel plate (20×20 cm). The silica gel containing the main band was scraped off and extracted with methanol–chloroform. Evaporation of the extract left a residue which after sublimation consisted of colorless prisms, m.p. 134–137°, $[\alpha]_D$ +9.7° (*c*, 1.76 in MeOH). In admixture with O-methylaspidoalbine (m.p. 127–131°) it melted at 128–135°. Its infrared spectrum was superimposable on that of O-methylaspidoalbine.

Aspidocarpine and O-Demethylaspidocarpine

The alcoholic extract of the bark was fractionated on a 200 g/200 ml kieselguhr/phosphate buffer (pH 5.2) column, using first ether and then chloroform as eluting solvents. Only traces of base were left on the column, which was stripped with ammoniacal chloroform.

Evaporation *in vacuo* of the ether eluate at room temperature left a yellow resin, which was dissolved in boiling benzene and decolorized with charcoal. The filtered cooled solution, left to stand overnight, deposited long pale yellow needles, m.p. 139–140°. After two recrystallizations from benzene the substance consisted of colorless needles, m.p. 149–150°. Chromatography on Whatman No. 1 paper with butanol as solvent showed the substance to be a mixture of two components. Recrystallization from aqueous isopropanol effected a separation and gave pure alkaloid III as colorless plates. The basic mixture recovered from the ether eluate after separation of alkaloid III was chromatographed on grade V neutral alumina. *n*-Hexane – chloroform (4:1) removed first a portion of unidentified material and then the major component of the mixture which, after crystallization from methanol, consisted of colorless prisms, m.p. 166–168°, identical (by melting point of the mixture and infrared spectra) with an authentic sample of aspidocarpine. Further elution of the chromatogram with chloroform removed another portion of unidentified material, and finally with chloroform containing 5% of methanol gave another quantity of alkaloid III.

[5]*This sample was kindly sent to us by Dr. C. Djerassi, to whom we express our gratitude for his cooperation.*

Alkaloid III, m.p. 156–158°, was soluble in aqueous sodium hydroxide but not in sodium carbonate. It gave a violet coloration with ferric chloride solution. A sample of the base was dried at 100° *in vacuo* and used for analysis. Found: C, 70.7; H, 7.96; N, 7.90. Calc. for $C_{21}H_{28}O_3N_2$: C, 70.79; H, 7.86; N, 7.86%. No methoxyl group was present. $[\alpha]_D^{25}$ +125° (*c*, 0.95 in chloroform). The infrared spectrum contained bands at 3320, 2520, 1635 with a shoulder at 1615 and a band at 1584 cm^{-1}. (Aspidocarpine produced infrared bands at 1635 and 1586 cm^{-1}.)

The alkaloid formed a hydrochloride which separated from ethanol–ether as colorless needles containing solvent of crystallization, m.p. 283° (decomp.). On drying at 120° *in vacuo* the salt lost 11% of its weight. The dried sample was used for analysis. Found: C, 64.8; H, 7.48; N, 7.00; Cl, 9.37. Calc. for $C_{21}H_{28}O_3N_2.HCl$: C, 64.18; H, 7.18; N, 7.13; Cl, 9.02%.

Methylation of Alkaloid III

A small sample of alkaloid III was methylated under the usual conditions with dimethyl sulphate and sodium hydroxide. The product of the reaction was sublimed *in vacuo* to remove colored impurities and then chromatographed on silica gel plates with the use of chloroform – *n*-hexane – diethylamine (10:20:3) as solvent. The main band, detected by spraying with water, was scraped off and extracted with chloroform–methanol. The filtered solution was evaporated and the residue crystallized from aqueous methanol, from which it separated as colorless prisms, m.p. 146—150°, $[\alpha]_D^{25}$ −95.4° (*c*, 1.18 in chloroform). It was identical with O-methylaspidocarpine in optical rotation ($[\alpha]_D^{25}$ −94° in chloroform), in melting point and that of the mixture, in plate chromatographic mobility, and in infrared absorption spectra.

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