ISOLATION AND STRUCTURE OF A NEW ALKALOID: ASPIDOCARPINE[1]

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ABSTRACT

From the root bark of *Aspidosperma megalocarpon* Muell. Arg. a new alkaloid, aspidocarpine, has been isolated. It has the empirical formula $C_{22}H_{30}O_3N_2$ and contains an N-acetyl, one methoxyl group, and one active hydrogen. The alkaloid, a phenolic monoacidic tertiary base, has been shown by its properties, those of its derivatives, and the spectroscopic data, to contain an N-acetyl-6-methoxy-7-hydroxydihydroindole moiety. Oxidation of aspidocarpine with chromic acid gave rise to a product identical in all respects with apoaspidospermine obtained from the similar oxidation of aspidospermine. Since the structure of aspidospermine is known, the foregoing results establish the structure of the alkaloid aspidocarpine.

The root bark of *Aspidosperma megalocarpon* Muell. Arg., a tree that grows in British Guiana,[3] has been found to contain alkaloids. They mostly occur in the petroleum ether extract, and largely consist (0.06% of dry weight) of one base which it is proposed to designate aspidocarpine. Its empirical formula derived from the analytical figures of the base and its salts is $C_{22}H_{30}O_3N_2$. Aspidocarpine melts at 168.5–169.5°, and determination of functional groups revealed that it contains one acetyl group, one methoxyl, one additional C-methyl besides that arising from the acetyl, and one active hydrogen.

The ultraviolet spectrum of aspidocarpine contains maxima at λ_{max} 227 mμ, log ε 4.42, and λ_{max} 263.5 mμ, log ε 3.92. It resembles that of aspidospermine and suggests that the new alkaloid, like aspidospermine, contains the N-acetyl-indoline chromophore. The spectrum of aspidocarpine was not affected by the presence of mineral acid, but was profoundly changed by the addition of sodium hydroxide.

In the infrared the spectrum of aspidocarpine contains no apparent hydroxyl absorption, but shows a peak at 1632 cm^{-1} which, although weak and at a low wave-number, must be indicative of the carboxyl of an N-acetyl group. Since, however, the base does not contain a carboxylic group, but contains an active hydrogen and is soluble in sodium hydroxide, since, further, it produces a pale olive-green color with ferric chloride, it must be phenolic. If the phenolic hydroxyl and the N-acetyl group were located sufficiently close to one another to form a strong hydrogen bond the effect in the infrared would shift the hydroxyl and carbonyl absorption to lower wave-numbers. Hence any hydroxyl absorption would be shifted to and covered by the CH stretching absorption region. The situation would thus parallel that observed in demethylaspidospermine (1). Evidence that this assumption is indeed correct has been obtained by blocking the hydroxyl function and thus removing the possibility of hydrogen bonding. Acetylation of the hydroxyl group was carried out smoothly in acetic anhydride and pyridine. The product, O-acetylaspidocarpine ($C_{24}H_{32}O_4N_2$) shows bands in the infrared at 1768 cm^{-1} and 1204 cm^{-1} characteristic of a phenolic O-acetyl (2) and another band at 1666 cm^{-1} due to the carbonyl of the N-acetyl group.

The phenolic hydroxyl of aspidocarpine did not react with diazomethane under the usual conditions, a common occurrence with strongly hydrogen-bonded phenols, but methylation was readily effected by the use of alkaline dimethylsulphate at room tempera-

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ture. The O-methylaspidocarpine ($C_{23}H_{32}O_3N_2$) thus produced showed a strong absorption band in the infrared at 1656 cm^{-1} due to the N-acetyl carbonyl. The ultraviolet spectra of both the O-acetyl and the O-methyl derivatives were not appreciably altered by sodium hydroxide. Thus when the phenolic hydroxyl is blocked and no hydrogen bonding is possible, the carbonyl absorption of the N-acetyl group in the infrared appears with its normal frequency and intensity.

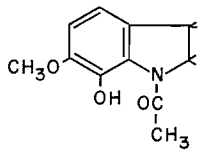
The N-acetyl group was characterized by hydrolysis of the alkaloid with sulphuric acid, steam-distillation of the reaction mixture, and spot-testing of the ethylamine salt of the distilled acetic acid on a paper chromatogram.

O-Methylaspidocarpine was hydrolyzed with 10% hydrochloric acid to O-methyl-deacetylaspidocarpine ($C_{21}H_{30}O_2N_2$). The infrared spectrum of this product no longer shows any carbonyl absorption, but contains a band at 3335 cm^{-1} attributable to an imino group. Treatment of the product with acetic anhydride and pyridine regenerates O-methylaspidocarpine, thus confirming the presence of an N-acetyl group in the alkaloid.

Treatment of aspidocarpine with hydrobromic acid produced an extremely unstable material which could not be crystallized. This material gives a blood red color with ferric chloride, which is consistent with an aminodihydric phenol. The material must be O-demethyl-N-deacetylaspidocarpine because on acetylation it is converted to O,O-diacetyl-O-demethylaspidocarpine ($C_{25}H_{32}O_5N_2$). The latter has an infrared absorption spectrum containing bands at 1777 and 1767 cm^{-1} (C=O) and 1204 cm^{-1} (C—O—) due to two phenolic O-acetyl groups, as well as a band at 1668 cm^{-1} attributable to the non-bonded carbonyl of the N-acetyl group. This observation places the original methoxyl group of aspidocarpine in the aromatic ring. In support of this conclusion it has proved possible to hydrolyze selectively the O-acetyl groups of O,O-diacetyl-O-demethylaspidocarpine and isolate pure O-demethylaspidocarpine, $C_{21}H_{28}O_3N_2$, which has the characteristics of a dihydric phenol. In the infrared spectrum of this compound, as expected, the carbonyl absorption shows the effect of hydrogen bonding, and the hydroxyl region contains an absorption band attributable to the non-hydrogen-bonded hydroxyl group. Treatment of O-demethylaspidocarpine with diazomethane did not affect the original hydroxyl, which had already been shown to resist methylation by this method, but methylated the second hydroxyl, thus regenerating aspidocarpine.

O-Demethylaspidocarpine behaves as an ortho-dihydric phenol as shown by the following observations. With ferric chloride it produces initially an intense blue-green color, which on addition of sodium carbonate, changes to a very deep red, a color sequence characteristic of catechols. In a series of tests on the compounds already described it was observed that coupling with diazotized sulphanilic acid occurred whenever either the hydroxyl or the indoline nitrogen was free, but did not occur when both were substituted. This indicates that the positions in the benzene ring para to these two functions are unsubstituted. Attempts to oxidize aspidocarpine or O-demethylaspidocarpine with periodate by the method of Adler and Magnusson (3) failed to give any crystallizable products, but produced color sequences indicative of the formation of an ortho-quinone. The condensation of either the presumed crude quinone with ortho-phenylenediamine, or of O-demethylaspidocarpine with phosgene led invariably to amorphous products of doubtful composition. The product of the reaction with phosgene, however, showed in the infrared a peak at 1835 cm^{-1}, a value indicative of the formation of a cyclic carbonate. It is of some interest that O-methylaspidocarpine gives no color in the brucine test with

nitric acid and acetic acid, an observation in accordance with the model experiments of Lyons, Perkin, and Robinson (4), whereas aspidocarpine and O-demethylaspidocarpine both give a deep red color different from the characteristic brucinequinone color. The best evidence in favor of the ortho arrangement of the hydroxyl groups in O-demethylaspidocarpine came from an application of Jurd's method (5), which is based on the effect of buffered boric acid on the ultraviolet spectrum of a compound. The ultraviolet spectrum of O-demethylaspidocarpine shows a dramatic change in the presence of buffered boric acid and parallel experiments show that catechol undergoes a similar change while the other dihydroxybenzenes are unaffected. The spectrum of aspidocarpine is unaffected by buffered boric acid. The sum of the evidence therefore strongly favors partial structure I as representing rings A and B of aspidocarpine. The nuclear magnetic resonance evidence, discussed later, also supports this conclusion.



I

The close similarity between the chemistry of aspidocarpine and that of aspidospermine, as well as in the spectral properties of both alkaloids, is quite striking. The relationship in rings A and B has already been demonstrated. In the study of the saturated moiety of the molecule it has been found that the reactions of aspidocarpine parallel exactly those of aspidospermine including the rather remarkable rearrangements known to take place in the latter (6).

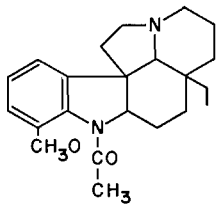
In line with aspidospermine (6, 7) aspidocarpine showed a high analytical N-methyl value (76% of calculated figure for one N-methyl) which later proved spurious, and still in keeping with other *Aspidosperma* alkaloids, O-methylaspidocarpine gave an N-methyl value considerably lower than that obtained with the alkaloid (24% of calculated value for one such group).

Aspidocarpine-N(*b*)-methiodide was readily prepared in dimethylformamide. It did not undergo the Hofmann degradation, however, but gave back aspidocarpine.

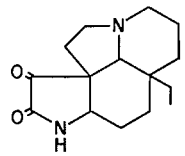
The von Braun degradation of aspidocarpine also produces a significant parallel with the behavior of aspidospermine in this reaction (6). Treatment of aspidocarpine with cyanogen bromide in chloroform (the reaction failed in benzene) gives rise to aspidocarpine bromocyanamide, $C_{23}H_{30}O_3N_3Br$, which exhibits the usual characteristics of this class of compounds, except that the normal solvolytic completion of the von Braun degradation fails since the bromocyanamide reverts to aspidocarpine even on reflux with ethanol.

These similarities between aspidospermine and aspidocarpine, coupled with their botanical relationship, suggested the possibility that they might differ only insofar as ring A was concerned. Ewins (8) had oxidized aspidospermine with chromic acid and had obtained a product, m.p. 193°, to which he tentatively assigned the structure of a keto-lactam (apoaspidospermine) resulting from the oxidative degradation of the

aromatic ring. The structure of aspidospermine (II) recently determined by X-ray crystallography[4] (9) leads to structure III for apoaspidospermine,



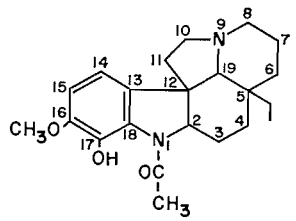
II



III

which should also be the product of the similar oxidation of aspidocarpine. Ewins' analytical figures were not in agreement with this structure but in our hands the oxidation of aspidospermine with chromic acid yielded a product, m.p. 224–225°, which gave analytical figures in excellent agreement with those required by III ($C_{15}H_{22}O_2N_2$). Both the infrared and ultraviolet spectra of the oxidation product were consonant with the presence of a five-membered keto-lactam in the molecule. This product was most probably identical with Ewins' apoaspidospermine, although it was purer as shown by the higher melting point. Aspidocarpine when oxidized with chromic acid yielded a product which had the same optical rotation and the same melting point as apoaspidospermine, alone or in admixture. The infrared spectra and the X-ray powder diagrams of both products were exactly superimposable.

The identity of the oxidation products of aspidocarpine and aspidospermine establishes the structure of the saturated moiety of the former, and this, together with the demonstration of the presence of a hydroxymethoxy-N-acetyl-indole in the molecule, affords conclusive proof that the structure of aspidocarpine is



IV

represented by formula IV. The assignment of the structure of the saturated part of the molecule depends on the X-ray crystallographic analysis of aspidospermine (9), and it follows from the identity of the oxidation products obtained from both alkaloids.

Aspidocarpine has proved very suitable for examination by nuclear magnetic resonance[5] especially since the spectrum can be compared with that of aspidospermine, the structure of which has been conclusively established (9, 10).

[4]*We acknowledge with thanks the courtesy of Professor N. S. Nyburg, who communicated to us the structure of aspidospermine in advance of his publication.*

[5]*Spectra were obtained with a Varian Associates high resolution nuclear magnetic resonance spectrometer operating at 60 Mc/s frequency. Chloroform was employed both as a solvent and as an internal reference; its proton resonance was assigned a value of zero c.p.s. and the scale was fixed from the position of the associated* C^{13} *(natural abundance) peaks which were shown to have a value of ±105 c.p.s.*

The significant features of the spectrum of aspidocarpine are:

(1) A single sharp peak at −215 c.p.s. with area equivalent to one proton. This is attributed to the internally hydrogen-bonded phenolic OH group.

(2) A triplet with strong central peak at 44 c.p.s. This multiplet produced by the aromatic protons is of the AB type (11) with a very small chemical shift and $J = 9$ c.p.s. The total area is equivalent to two protons.

(3) A quartet at 193, 198, 202, and 208 c.p.s. with a total area equivalent to one proton. This is attributed to the proton at C2. The structure shows the effect of splitting by the adjacent methylene protons and demonstrates the rigidity of ring C.

(4) A single sharp peak at 211 c.p.s. of area equivalent to three protons. This is attributed to the protons of the methoxyl group.

(5) A multiplet at 247, 255, 258, and 263 c.p.s. of which the 258 c.p.s. peak is the strongest and sharpest. These signals are more difficult to assign to particular protons, but are probably to be associated with the protons on carbons adjacent to the nitrogen at position 9. From the area under the curve, two or at most three protons are involved with this resonance. Since ring size is known to affect the position of proton resonance in saturated heterocyclic systems (12) it would be expected that the signals of the protons at C8 would be separated from those at C10. The most likely situation is that the protons at C10 (in a five-membered ring) are involved with this multiplet while the C8 protons (in a six-membered ring) appear in the general absorption above 300 c.p.s. The sharp peak at 258 c.p.s. may be part of the C10 resonance or, if the total area does in fact represent three protons, it may be caused by the proton at C19, which is common to a five-membered and a six-membered ring and is isolated from protons which could cause splitting.

(6) A single sharp peak at 303 c.p.s. with an area equivalent to three protons. It is attributed to the protons of the N-acetyl group.

(7) A sharp peak at 400 c.p.s. with an area corresponding to about two protons, but the peak is probably associated with some of the adjacent structure and is attributed to the methyl protons of the ethyl group.

A comparison of the spectrum of aspidospermine with that of aspidocarpine shows almost all the expected relationships.

(1) The OH peak at −215 c.p.s. is absent.

(2) The aromatic protons (now three) show a multiplet of the ABC type centered on 32 c.p.s., but a complete analysis is not possible since only nine separate peaks are observed.

(3) The quartet associated with the proton at C-2 now appears at 165, 171, 175, and 182 c.p.s. The shift from the corresponding values in aspidocarpine is associated with the nature of the N-acetyl group. In aspidocarpine the carbonyl of this group is hydrogen-bonded with the C17 hydroxyl and this certainly pulls the oxygen away from the C2 proton. In aspidospermine, hydrogen bonding does not occur and it can readily be seen from models that the acetyl group will take up a less hindered conformation.[6] Since a shift occurs the important deduction that can be drawn is that the assignment of the quartet to the C2 proton resonance is confirmed.

The remainder of the spectrum of aspidospermine is essentially the same as that of aspidocarpine except that the N-acetyl protons appear at 8 c.p.s. lower in the latter, an

[6]*There are two less hindered conformations that can be taken up by the acetyl group. In one conformation the carbonyl and the* C2—H *bond have a cis relationship but are trans in the other. A cis relationship would cause a shift to higher frequency and since the observed shift is to lower frequency the trans relationship is more probable. Stereochemical predictions from scale models are in agreement with this.*

effect probably produced by the hydrogen bonding with the acetyl carbonyl in aspidocarpine.

The spectrum of O-methylaspidocarpine has also given interesting information. Since the phenolic hydroxyl is blocked, hydrogen bonding is excluded in this derivative and the spectrum is very similar to that of aspidospermine except that there are two methoxyl peaks (210 and 215 c.p.s.) and the aromatic proton region shows a complete AB type (11) quartet centered on 39 c.p.s. with an area equivalent to two protons (chemical shift = 11.5 c.p.s. and $J = 8$ c.p.s.). The quartet associated with the C2 proton (166, 172, 176, 182 c.p.s.) is essentially the same as in the spectrum of aspidospermine. The relationship between the spectra in the aromatic region of aspidocarpine and O-methylaspidocarpine affords supporting evidence for the assignment on chemical grounds of substituents to positions 16 and 17. Since the oxygen function at position 17 is considered to be conclusively established, the original methoxyl group must be located at one of the three positions (14, 15, or 16). Position 15 meta to position 17 is excluded because the spectrum requires two adjacent protons. If in O-methylaspidocarpine the original methoxyl were at position 14 the protons at 15 and 16 would have almost identical environment, and a very small chemical shift would be expected. In fact the chemical shift in the O-methylaspidocarpine spectrum is greater than in the spectrum of aspidocarpine and the methoxyl must therefore be placed at position 16. The small chemical shift for the aromatic protons in aspidocarpine is explainable by the fact that the effect of a hydroxyl group on a proton para to it (i.e., at position 14) is almost the same as the effect of a methoxyl group (position 16) on a proton at its ortho position (i.e. position 15).

Conroy and his co-workers (6) have made an analysis of the spectrum of aspidospermine using a 40 Mc/s instrument, and while the major features of their spectrum correspond to those of ours (after the appropriate conversions of scale, etc.) there are several important differences. The peaks that we have assigned to the C2 and C10 protons are not easily seen in their chloroform solution spectrum, but are more evident when they used carbon tetrachloride as solvent. The peaks close to the N-acetyl and methoxyl signals which they assign to the CH_2 groups and the CH adjacent to the nitrogen do not appear in our spectrum.

EXPERIMENTAL

Melting points were determined on a Kofler micro hot stage. The infrared spectra were obtained with a Perkin–Elmer model 21 spectrophotometer, and the absorption is reported in wave numbers followed by a bracketed (m) or (s) to indicate medium or strong absorption. The ultraviolet spectra unless otherwise stated were determined with a Beckman model DU spectrophotometer.

Isolation of Aspidocarpine

The root bark of *Aspidosperma megalocarpon* Muell. Arg. (83.2 kg) was ground to a powder and percolated with petroleum ether (b.p. 95°–110°). The petroleum ether extract was concentrated under reduced pressure and the concentrate deposited the crude alkaloid in crystalline form. Aspidocarpine was purified by recrystallization from heptane and from methanol from which it separated as colorless prisms (50 g), m.p. 168.5–169.5°, $[\alpha]_D^{25}$ +140° (*c*, 2.3 in chloroform), pK_a, 6.55 (by titration with *p*-toluenesulphonic acid). Found: C, 71.18, 71.13; H, 8.10, 8.11; N, 7.52; act. H, 0.26; CH_3CO, 10.06; C—CH_3, 6.34; OCH_3, 8.28, 8.29; N—CH_3, 3.09 (spurious result); mol. equiv. 371, 374 (acid titration). Calc. for $C_{22}H_{30}O_3N_2$; C, 71.32; H, 8.16; N, 7.56; 1 act. H, 0.27;

1 CH_3CO, 11.61; 2C.CH_3, 8.12; 1 OCH_3, 8.38; mol. wt. 370. Infrared in chloroform: 1632, 1603, 1582 cm^{-1}, in nujol: 1637, 1605, 1587 cm^{-1}. Ultraviolet spectrum in 95% ethanol: λ_{max}, 228 mμ, log ϵ 4.42, 263.5 mμ, log ϵ 3.92, λ_{min} 249 mμ, log ϵ 3.80; after addition of a drop of aqueous sodium hydroxide: λ_{max} 224 mμ, log ϵ 4.29, 238 mμ, log ϵ 4.29, 308 mμ, log ϵ 3.65.

Aspidocarpine perchlorate, prepared in the usual manner separated from methanol as colorless needles, m.p. 280–282° (decomp.). Found: C, 56.38; H, 6.29; N, 5.93. Calc. for $C_{22}H_{30}O_3N_2.HClO_4$: C, 56.11; H, 6.64; N, 5.95%. Ultraviolet spectrum in 95% ethanol: λ_{max} 227 mμ, log ϵ 4.28, 262 mμ, log ϵ 3.82; λ_{min} 250 mμ, log ϵ 3.68.

Aspidocarpine hydrochloride crystallized from ethyl acetate–ethanol as colorless prismatic needles, m.p. 224–230° (decomp.). Found: C, 64.47; H, 7.52. Calc. for $C_{22}H_{30}O_3N_2.HCl$: C, 64.93; H, 7.63%.

The hydrobromide separated from methanol as prismatic needles m.p. 288–290°. Found: C, 57.82; H, 6.68. Calc. for $C_{22}H_{30}O_3N_2.HBr.O.5H_2O$: C, 57.38; H, 7.00%.

Aspidocarpine hydriodide crystallized in rectangular plates from methanol, m.p. 272–274° (decomp.). Found: C, 52.17; H, 6.09. Calc. for $C_{22}H_{30}O_3N_2.HI.0.5H_2O$: C, 52.08; H, 6.35%.

O-Acetylaspidocarpine

Aspidocarpine (281 mg) was dissolved in acetic anhydride (3 ml) containing pyridine (0.8 ml) and allowed to stand at room temperature for 6 hours. The solution was evaporated to dryness under reduced pressure, the residue dissolved in benzene and chromatographed on neutral alumina. Careful elution with benzene and evaporation of the eluate gave a crystalline product which separated from hexane–ether as colorless prisms, m.p. 165–167°, $[\alpha]_D^{25}$ −8.4° (*c*, 0.81 in chloroform). Found: C, 69.90; H, 7.78; CH_3CO, 20.25. Calc. for $C_{24}H_{32}O_4N_2$: C, 69.88; H, 7.82; CH_3CO (one OAc+one NAc) 20.86%. Infrared in chloroform: 1768 (s), 1666 (s) cm^{-1}. Ultraviolet (95% ethanol; Cary model 11M): λ_{max} (log ϵ) 223 mμ (4.53), 253 mμ (3.98), 292 mμ (3.61); λ_{min} (log ϵ), 240 mμ (3.89), 272 mμ (3.28). The spectrum was not appreciably affected by the presence of base.

O-Methylaspidocarpine

Aspidocarpine (1.040 g) was dissolved in dilute hydrochloric acid (20 ml) and the solution made basic by the cautious addition with stirring of 20% aqueous sodium hydroxide. A precipitate was first formed which redissolved after the solution had become basic. Methylation was carried out with methyl sulphate (5 ml) in the usual manner. The product which separated as the reaction proceeded was filtered off and washed with water. After two crystallizations from aqueous methanol it consisted of prismatic needles (0.752 g) m.p. 152–154° $[\alpha]_D^{25}$ −94° (*c*, 1.15 in chloroform). Found: C, 71.44; H, 8.10; CH_3O, 16.58; CH_3N, 0.94 (spurious). Calc. for $C_{23}H_{32}O_3N_2$: C, 71.84; H, 8.39; $2CH_3O$, 16.14%. pK_a 6.9 (titration with *p*-toluenesulphonic acid in 50% ethanol).

Infrared (chloroform): 1660 (sh), 1645 (s) cm^{-1}; in carbon tetrachloride, 1663 (s) cm^{-1}; in nujol, 1656 (s) cm^{-1}. Ultraviolet in 95% ethanol: λ_{max} (log ϵ) 224 mμ (4.60), 252 mμ (4.07), 288 mμ (3.44), λ_{min} (log ϵ), 244 mμ (4.03), 279 mμ (3.41). The spectrum was not appreciably affected by base.

O-Methyldeacetylaspidocarpine

O-Methylaspidocarpine (107 mg) was dissolved in 10% hydrochloric acid (5 ml) and the solution refluxed in an atmosphere of nitrogen for 3 hours. The cooled solution was

made basic with solid sodium carbonate and immediately extracted with chloroform. After drying (sodium sulphate) the extract was evaporated to dryness and the residue dissolved in aqueous methanol from which it crystallized as white needles (50 mg), m.p. 151–152°. Recrystallization raised the melting point to 153–154°. $[\alpha]_D^{25}$ −4.9° (*c*, 1.03 in chloroform). pK_a 7.8 (titration with *p*-toluenesulphonic acid in 50% ethanol). Found: C, 73.95; H, 8.83; N, 8.65. Calc. for $C_{21}H_{30}O_2N_2$: C, 73.64; H, 8.83; N, 8.18%. Infrared in nujol 3335 cm^{-1}. Ultraviolet in 95% ethanol: strong end absorption, λ_{infl} 240 mμ, log ε 3.82, λ_{min} 268 mμ, log ε 2.97, λ_{max} 293 mμ, log ε 3.49. The spectrum was not appreciably affected by acid.

Treatment of a sample of O-methyldeacetylaspidocarpine with acetic anhydride and pyridine reconverted it to O-methylaspidocarpine in about 50% yield.

O,O-Diacetyl-O-demethylaspidocarpine

Aspidocarpine (731 mg) was refluxed with constant boiling hydrobromic acid (10 ml) in an atmosphere of nitrogen for 1 hour. The solution was evaporated to dryness under reduced pressure and the residue heated on the steam bath with acetic anhydride (15 ml) in an atmosphere of nitrogen for 3 hours. The solution was evaporated to dryness under reduced pressure and the residue dissolved in dilute aqueous sodium carbonate. This solution was extracted with ether and the extract washed with a little water and dried (sodium sulphate). Evaporation of the ether yielded a residue (757 mg) which crystallized from hexane–acetone as yellowish microcrystals (510 mg), m.p. 140–144°. After treatment with charcoal and recrystallization from the same solvent, the product consisted of colorless platelets, m.p. 144–145°, $[\alpha]_D^{25}$ +9.8° (*c*, 1.33 in chloroform). Found: C, 68.21; H, 7.19; CH_3CO, 30.36. Calc. for $C_{25}H_{32}O_5N_2$: C, 68.16; H, 7.32; $3CH_3CO$, 29.31%. Infrared in chloroform: 1777 (sh), 1767 (s), 1668 (s) cm^{-1} (in nujol, 1204 cm^{-1}).

O-Demethylaspidocarpine

The material was prepared first by controlled hydrolysis of O,O-diacetyl-O-demethylaspidocarpine but it was found more convenient to start with aspidocarpine and carry out the whole preparation in the same flask without isolating the intermediates in pure form.

Aspidocarpine (1.043 g) was treated successively with hydrobromic acid and acetic anhydride as described above for the preparation of the diacetyl derivative. After removal of the acetic anhydride, the crude residue was dissolved in methanol (25 ml) to which was added 20% aqueous sodium hydroxide (2.5 ml). The solution was refluxed in an atmosphere of nitrogen for 20 minutes and then evaporated under reduced pressure to a volume of about five milliliters. Crushed ice (ca. 5 g) was added, the solution made just acid with 5% sulphuric acid, then brought to pH 8 with aqueous sodium carbonate and finally thoroughly extracted with chloroform. The extract was dried (sodium sulphate) and the solvent removed by evaporation. The residue, a brown solid (0.845 g) was dissolved in a mixture of ether and benzene and passed through a short column of silica gel which was then eluted with a mixture of benzene (4 parts) and ether (1 part). Evaporation of the eluate yielded a colorless solid (0.676 g) which, on crystallization from aqueous methanol, formed needles, m.p. 130° with an apparent phase change and partial melting at 92–94°. Recrystallization from benzene produced colorless tablets, m.p. 130°, with softening above 125°. After drying *in vacuo*, found: C, 70.91; H, 8.17; N, 7.79. Calc. for $C_{21}H_{28}O_3N_2$: C, 70.76; H, 7.92; N, 7.86%. $[\alpha]_D^{25}$ +121° (*c*, 0.81 in chloroform). Infrared (nujol), 3330 (m), 1634 (s) cm^{-1}. Ultraviolet (pure ethanol): λ_{max} (log ε) 227 mμ (4.41),

262 mμ (4.01), λ_{min} (log ε) 248 mμ (3.86). In the presence of buffered boric acid: strong end absorption, λ_{infl} (log ε) 258 mμ (4.08), λ_{max} (log ε) 290 mμ (3.56).

For the determination of the ultraviolet spectra in the presence of buffered boric acid a stock solution of O-demethylaspidocarpine (2.0 mg) in pure ethanol (10 ml) was prepared. Simple dilution of the stock solution afforded the solution used in determining the spectrum of the untreated substance. For the spectrum in the presence of buffered boric acid, the stock solution (1 ml) was added to a saturated solution of boric acid in ethanol (2 ml) and diluted to 10 ml with ethanol. Excess anhydrous sodium acetate was added, and the mixture was thoroughly shaken and allowed to stand for 20 minutes before use.

Treatment of O-demethylaspidocarpine in methanol with an ethereal solution of diazomethane reconverted it to aspidocarpine in 60% yield.

Aspidocarpine-N(b)-methiodide

Aspidocarpine (258 mg) was dissolved in dimethylformamide (5 ml) containing methyl iodide (3 ml) and heated under reflux on a steam bath for 4 hours. The solution was evaporated to dryness on a rotary evaporator and the yellow residue crystallized from methanol from which it separated as yellowish crystals (170 mg), m.p. 280–284° (decomp.). Recrystallization from the same solvent gave colorless rectangular plates, m.p. 288–290° (decomp.). Found: C, 52.93; H, 6.26. Calc. for $C_{22}H_{30}O_3N_2.CH_3I.O.5H_2O$: C, 52.98; H, 6.57%.

Aspidocarpine-N(*b*)-methiodide (34 mg) was refluxed for 30 minutes with a solution of potassium hydroxide (70 mg) in 95% ethanol (5 ml) in an atmosphere of nitrogen. The solution was evaporated to dryness under reduced pressure and the yellowish residue dissolved in water (5 ml). The aqueous solution was thoroughly extracted with ether, the extract washed with a little water, dried (sodium sulphate), and evaporated to dryness. The residue was a slightly discolored crystalline solid (17 mg) which, on recrystallization, proved identical with an authentic sample of aspidocarpine.

von Braun Degradation: Aspidocarpine Bromocyanamide

Aspidocarpine (215 mg) was refluxed with cyanogen bromide (416 mg) in chloroform (10 ml) for 24 hours. Evaporation of the solution left a white residue which was triturated with ethyl acetate. The insoluble fraction (70 mg) proved to be aspidocarpine hydrobromide. The ethyl acetate solution when concentrated deposited a colorless crystalline solid (133 mg), m.p. 167–169°, which formed needles, m.p. 170–171° on recrystallization from methanol. Found: C, 58.45; H, 6.33; N, 8.93. Calc. for $C_{23}H_{30}O_3N_3Br$: C, 57.98; H, 6.35; N, 8.81%. Infrared (nujol): 2218(m), 1633(s) cm^{-1}.

In a subsequent preparation, the alkaloid hydrobromide was removed by dissolving the crude mixture in chloroform and chromatography on a short column of neutral alumina.

Aspidocarpine bromocyanamide (73 mg) was refluxed for 6 hours with 95% ethanol (9 ml) in an atmosphere of nitrogen. The solution was concentrated to a small volume under reduced pressure, diluted with a little water, made basic with solid sodium carbonate, and extracted with chloroform. The extract, after drying over sodium sulphate, was chromatographed on a short column of neutral alumina and eluted with chloroform. The eluate yielded a solid product (60 mg) which, on crystallization from methanol, formed colorless prisms identical in every respect with aspidocarpine.

Chromic Acid Oxidation

(a) Aspidocarpine

The alkaloid (1.131 g) was dissolved in water (17 ml) containing sulphuric acid (3 ml) and heated on a steam bath. Chromium trioxide (1.8 g) dissolved in a little water was cautiously added over a period of about one hour. Heating was continued for a further hour and the hot solution subsequently made basic with hot aqueous barium hydroxide and filtered. The yellow filtrate was exhaustively extracted with chloroform and the dried (sodium sulphate) extract evaporated. A semisolid residue was left (0.169 g) which was dissolved in benzene, chromatographed on neutral alumina, and crystallized in a mixture of hexane and acetone from which it separated as yellowish microcrystals (76 mg), m.p. 222–224°. Recrystallization from the same solvent gave pale yellowish rods, m.p. 224–225°, $[\alpha]_D^{25}$ −132° (*c*, 1.03 in chloroform). Found: C, 68.87; H, 8.52; N, 10.63. Calc. for $C_{15}H_{22}O_2N_2$: C, 68.67; H, 8.45; N, 10.68%. Infrared (chloroform): 3410 (w), 1762 (s), 1728 (vs) cm^{-1}. Ultraviolet (95% ethanol): λ_{min} 227 mμ log ε 3.31, λ_{max} 252 mμ log ε 3.55, 336 mμ, ε 59. In the presence of a drop of dilute aqueous potassium hydroxide: λ_{min} 234 mμ log ε 3.28, λ_{max} 268 mμ log ε 3.48 (longer wave-length absorption not measured).

(b) Aspidospermine

Aspidospermine obtained from Inland Alkaloid Co., and recrystallized (1.020 g) was treated with chromic acid as described above, except that heating was continued for 8 hours. The crude product (0.105 g) proved more difficult to purify than the oxidation product of aspidocarpine. The crystalline product, m.p. 223–225°, has $[\alpha]_D^{25}$ −128° (*c*, 0.47 in chloroform). Its infrared spectrum contained the same peaks as that of the oxidation product of aspidocarpine and the X-ray diffraction patterns of both compounds were identical.

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