886. *Alkaloid Studies. Part XLVIII.*[1] *The Structure of Apparicine, a Novel Aspidosperma Alkaloid*

By J. A. Joule, H. Monteiro, L. J. Durham, B. Gilbert, and Carl Djerassi

The *Aspidosperma* alkaloid apparicine is shown by nuclear magnetic resonance (n.m.r.) and mass spectral data as well as chemical transformations to possess the structure (IX). The possible biogenetic relationship of this novel structural type to uleine (I) is discussed.

(−)-Apparicine has been isolated [2] from four *Aspidosperma* species (A. *olivaceum* Mull-Arg., A. *eburneum* Fr. All., A. *multiflorum* A. DC., and A. *gomezianum* A. DC.) and the enantiomeric (+)-apparicine from a fifth (A. *Dasycarpon* A. DC.). Analyses of the akaloid show the presence of one *C*-methyl group and suggest $C_{18}H_{20}N_2$ as a molecular formula. The mass spectrum (Figure 1) exhibits a molecular ion at 264 mass units, confirming the

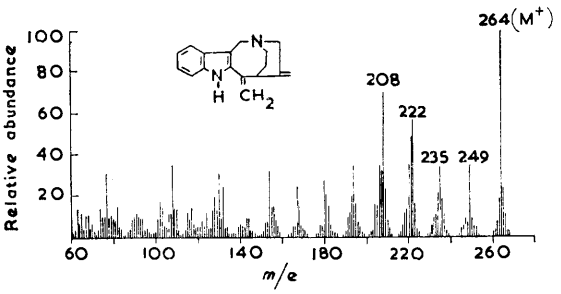


Figure 1. Mass spectrum of apparicine

molecular formula, and a fragmentation pattern strikingly similar to that [3] of uleine (I),[4] suggesting a close similarity between the two bases, apparicine having two fewer hydrogen atoms than uleine. The ultraviolet spectra of apparicine and uleine [5] are very similar. Their n.m.r. spectra both show indolic *N*-hydrogen signals (7·95 and 8·67 δ), signals for four aromatic protons (6·9—7·5 and 7·0—7·7 δ) and two exocyclic methylene proton singlets (5·24, 5·37, and 4·98, 5·28 δ, respectively). All these close similarities, together with the co-occurrence of the two alkaloids,[2] lead to the assignment of the uleine chromophore (II) to apparicine.

[1] Part XLVII, M. Ohashi, J. A. Joule, B. Gilbert, and C. Djerassi, *Experientia*, 1964, **20**, 363.

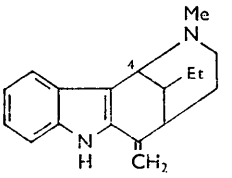
[2] For the isolation of apparicine and the structures of co-occurring bases see B. Gilbert, A. P. Duarte, Y. Nakagawa, J. A. Joule, S. E. Flores, J. A. Brissolese, J. Campello, E. P. Carrazoni, R. J. Owellon, E. C. Blossey, K. S. Brown, jun., and C. Djerassi, *Tetrahedron*, 1965, **21**, 1141, as well as ref. 1.

[3] J. A. Joule and C. Djerassi, *J.*, 1964, 2777.

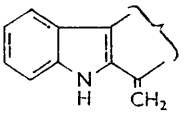
[4] J. Schmutz, F. Hunzicker, and R. Hirt, *Helv. Chim. Acta*, 1957, **40**, 1189; Büchi and Warnhoff, *J. Amer. Chem. Soc.*, 1959, **81**, 4433.

[5] N. Neuss, "Physical Data of Indole and Dihydroindole Alkaloids," vol. I, Eli Lilly and Co., Indianapolis, Indiana.

The 100 Mc./sec. n.m.r. spectrum of apparicine (Figure 2), unlike that of uleine, contains neither an *N*-methyl singlet nor a *C*-ethyl triplet, but does exhibit a three proton, finely split doublet ($J = 8$ c./sec., $J = 1\frac{1}{2}$ c./sec., the cause of the fine splitting will be discussed later in this Paper) at 1·47 δ. The major splitting was shown, by decoupling to be due to



(I)



(II)

an olefinic proton (broadened quartet centred at 5·24 δ, $J = 8$ c./sec., partially hidden under the exocyclic methylene proton signals). Since this combination of signals is highly characteristic, it can be concluded that apparicine contains an ethylidene grouping. Instead of the *one* proton doublet (4·11 δ, $J = 3$ c./sec.) shown by the C-4 hydrogen of uleine, which because it is both benzylic and adjacent to the basic nitrogen has a characteristic δ value, there appears in the spectrum of apparicine, a *two* proton AB quartet (4·27, 4·47 δ, $J = 18$ c./sec.). Thus since the basic nitrogen of apparicine was shown to be tertiary by the failure to obtain ether-soluble material on treatment of apparicine methiodide with aqueous potassium carbonate, a partial structure (III) can be set down

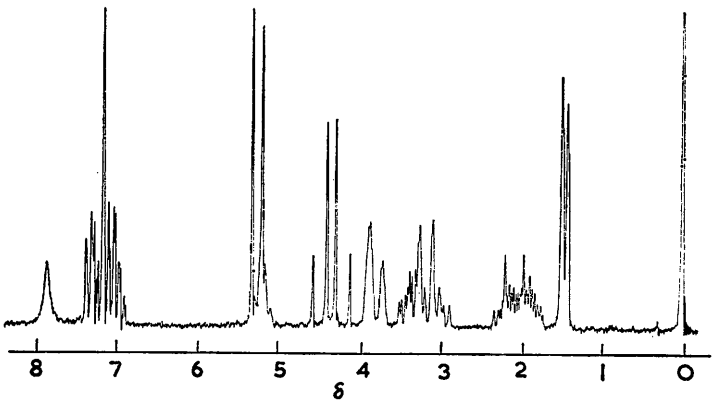
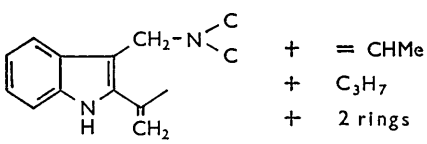


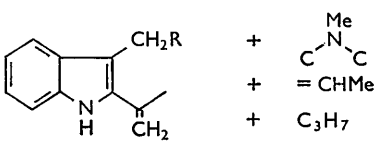
FIGURE 2. Apparicine: 100 Mc. n.m.r. spectrum

for apparicine, in which the carbon atom, C-6, joining the β-position of the indole nucleus to the basic nitrogen, N_b, carries *two* hydrogen atoms.

Further substantiation for the presence of the grouping β-indole $\cdot CH_2 \cdot N_b$ in apparicine is gained from the following two transformation products. It was shown [3] that uleine



(III)



(IV)

methiodide like gramine methiodide undergoes easy nucleophilic displacement of N_b^+ by nucleophiles such as methoxide, hydride or cyanide ions, at the benzylic carbon atom C-6. Apparicine methiodide underwent analogous reactions with sodium methoxide and lithium aluminium hydride (nucleophilic displacement of N_b^+ from C-6) to give N_b-methyl-6-methoxydihydroapparicine and N_b-methyldihydroapparicine, with part structures (IV; R =

OCH_3) and (IV; R = H), respectively. One of the two unspecified rings of the alkaloid is destroyed in the production of these two compounds.

The n.m.r. spectrum of N_b-methyl-6-methoxydihydroapparicine (IV; R = OCH_3), in contrast to that of the parent alkaloid, shows a two-proton singlet (4·61 δ) for the β-indole·CH_2·O grouping and a three-proton methoxyl singlet (3·38 δ). Thus the two hydrogen atoms situated on C-6 now become equivalent since the β-indole–C-6 bond, no longer constrained in a ring, is free to rotate. The downfield shift in the line position

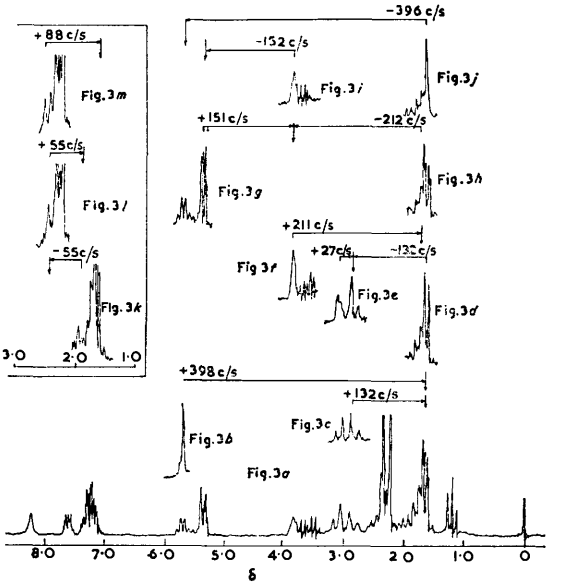


FIGURE 3. N_b-Methyldihydroapparicine: 100 Mc. n.m.r. spectrum and double resonance experiments

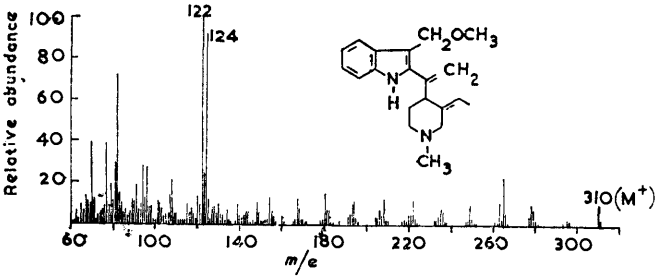


FIGURE 4. Mass spectrum of N_b-methyl-6-methoxydihydroapparicine

of the C-6 protons signal is in the same direction as that observed [3] in the analogous uleine derivative.

The n.m.r. spectrum (Figure 3, to be discussed in more detail later in this Paper) of N_b-methyldihydroapparicine (IV; R = H), contains no signal in the region 4·0—5·0 δ since the hydrogen atoms on C-6 though still benzylic are no longer adjacent to either oxygen or nitrogen. The spectrum does however contain a new three-proton singlet (2·33 δ) corresponding to the aromatic methyl grouping at the indole β-position.

It is noteworthy that in the ultraviolet spectra of the two compounds discussed above, the main band undergoes a hypsochromic shift of 11 mμ with respect to its position in the spectrum of the alkaloid. This must indicate a lesser degree of conjugation between the indole ring and the exocyclic double bond, since there is no doubt (see later discussion of the n.m.r. spectrum of N_b-methyldihydroapparicine) that the chromophore is intact. Compounds derived from uleine by fission of the C-4–N_b bond [3] in which the c-ring, presumably the main factor in constraining the methylenic double bond and the indole

system to a particular degree of coplanarity, is retained, show ultraviolet spectra identical with that of uleine itself.

The nature of the ring containing the basic nitrogen atom in N_b-methyl-6-methoxydihydroapparicine and N_b-methyldihydroapparicine can be deduced from their mass spectra (Figures 4 and 5) and from dehydrogenations of the parent alkaloid. Both mass spectra show major peaks at *m/e* 122 and 124. Since these peaks appear in the spectra of both compounds the ions must correspond to the aliphatic portions of the molecules,

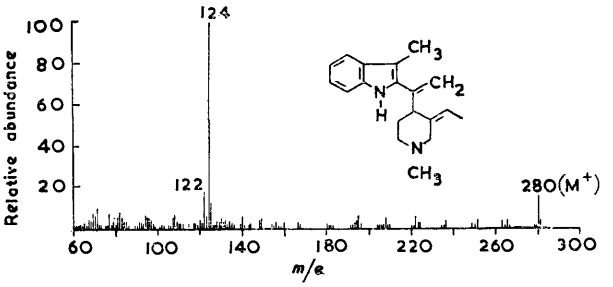
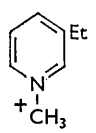


FIGURE 5. Mass spectrum of N_b-methyldihydroapparicine

the aromatic halves being differently substituted (IV; R = OMe and H, respectively). It may be concluded that the ions represent piperidine moieties substituted with an *N*-methyl and a two-carbon group, the ethylidene function. Such ions are well known to occur in the mass spectra of many indole alkaloids which contain a piperidine ring.[6] The *m/e* 122 species is best represented by (A), and the *m/e* 124 ion being in a lower oxidation state.



(A)

Pyridines could not be detected directly by vapour phase chromatography of the products of dehydrogenations of apparicine or N_b-methyldihydroapparicine (IV; R = H) with zinc dust or selenium. Oxidation of the crude dehydrogenation products with potassium permanganate however, gave nicotinic acid, identified by paper chromatography.[7] Dehydrogenation of apparicine with palladium–charcoal and subsequent permanganate oxidation gave a mixture of acids which were identified by paper chromatography as mainly pyridine-3,4-dicarboxylic acid and a smaller amount of nicotinic acid. The diester of the pyridine diacid was shown by mass spectrometric comparison with other pyridine diesters to be dimethyl pyridine-3,4-dicarboxylate.

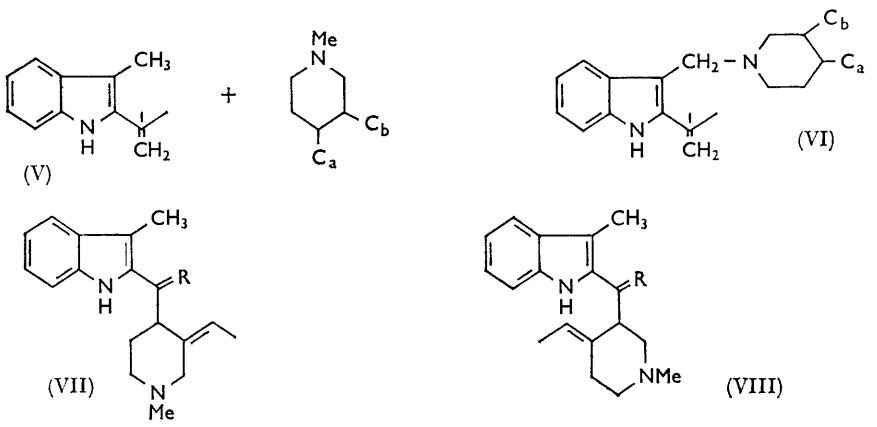
With this substantiation of the presence of a substituted piperidine moiety in N_b-methyldihydroapparicine and in apparicine itself, the part structure for the former can be extended to (V) and that for the latter to (VI). Structures (V) and (VI) contain all save one of the carbon atoms of the molecules and it remains only to establish which of the two carbon substituents (C_a or C_b) correspond to C-1, the other then necessarily representing the first carbon of the ethylidene side chain, the methyl of which is the remaining atom unaccounted for.

A decision between the two possibilities (VII; R = CH_2:; C_a = C-1) and (VIII; R = CH_2:; C_b = C-1) can be arrived at by an examination of the results of nuclear magnetic double resonance (n.m.d.r.) experiments on N_b-methyldihydroapparicine (Figures 3*b*—*m*).

In addition to the signals due to the aromatic ring (7·0—7·7 δ), the indole NH (8·17 δ), the N_b-methyl and aryl methyl (2·22 and 2·33 δ, respectively), the spectrum (Figure 3*a*) of N_b-methyldihydroapparicine shows signals characteristic of an exocyclic methylene (5·27 and 5·34 δ), and a vinyl quartet (5·65 δ) and methyl multiplet (1·67 δ) characteristic of an ethylidene group. Signals at 1·2 and 3·5 δ are due to an impurity in the sample.

[6] H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Massspectrometry: vol. I, Alkaloids," Holden-Day, Inc., San Francisco, 1964, ch. 3, 4, and 7.
[7] F. Kuffner, *Mh. Chem.*, 1955, **86**, 995; *ibid.*, 1963, **94**, 252.

Double resonance experiments were carried out using the method of Freeman [8] as modified by Johnson.[9] The decoupled signals (Figures 3*b*—*m*) are reproduced above the spectrum (Figure 3*a*), with horizontal arrows running to the site of irradiation



(indicated by vertical arrows). The observed differences in frequencies, which approximate the chemical shift differences (no correction applied) are given on the horizontal arrows. A positive number indicates that the site of irradiation is at a higher field and a negative number that it is at a lower field.

The presence of an ethylidene group was confirmed by irradiation of the methyl signal at 1·67 δ which caused the collapse of the quartet at 5·65 δ to a singlet (Figure 3*b*). Conversely the methyl multiplet was collapsed by irradiation of the vinyl proton (Figure 3*j*). The methyl multiplet was sharpened to a doublet by irradiation of the signal at 2·87 δ (Figure 3*d*). The interaction was checked by irradiation of the methyl group and observing the signal at 2·87 δ increase its amplitude to match the signal at 3·08 δ (Figure 3*c*). The degree of perturbation and the magnitude of the coupling constant ($J = 13$ c./sec.) of the latter two signals suggests they are due to a pair of geminal protons. The coupling was verified by n.m.d.r. (Figure 3*e*). The chemical shifts of this geminal pair indicate the presence of another electronegative group, either nitrogen or another double bond. The absence of a more complex coupling pattern also precludes the presence of a methylene (·CH_2·) group. From these data it is deduced that the molecule contains the part structure $CH_3CH{:}C{\cdot}CH_2X$, where X is either nitrogen or a double bond.

A second series of decoupling interactions established by n.m.d.r. techniques involves the exocyclic methylene group (signal at 5·34 and 5·27 δ). The multiplicity of both these signals was reduced simultaneously upon irradiation at a frequency corresponding to the proton signal at 3·82 δ (Figure 3*g*). The chemical shift of the latter requires the presence of at least one more electronegative group (nitrogen or a double bond). Coupling to the exocyclic methylene was confirmed (Figure 3*i*) and coupling to proton(s) in the vicinity of the ethylidene methyl established (Figure 3*f*) by appropriate irradiation. That the latter interaction was in fact one with signals in the vicinity of the methyl group but not with the methyl group itself was shown by irradiating the signal at 3·82 δ (Figure 3*h*).

Further n.m.d.r. experiments showed that the signals in the vicinity of the *N*-methyl signal (2·1—2·75 δ) are coupled with ones in the region (1·5—2·1 δ) of the ethylidene methyl (Figures 3*k*, *l*, *m*). The chemical shifts are compatible with a pair of methylene groups, one attached to a saturated carbon and one to an electronegative group (either nitrogen

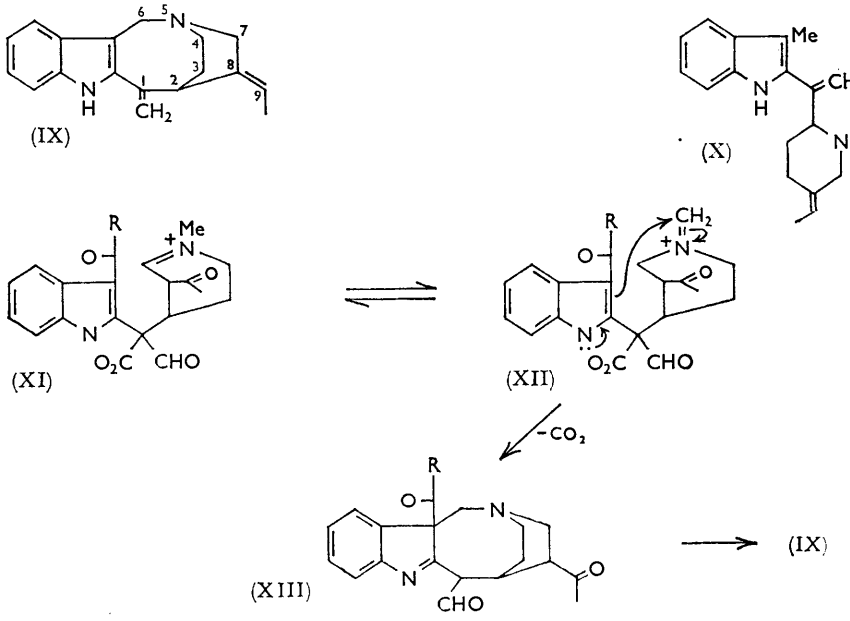
[8] R. Freeman, *J. Mol. Phys.*, 1960, **3**, 435; R. Freeman and D. H. Whiffen, *Proc. Phys. Soc.*, 1962, **79**, 794.

[9] L. F. Johnson, " Varian Tech. Information Bull. III," 1962, **3**, 5.

or a double bond). From these data the sequence $CH_2{:}C{\cdot}CH(Y){\cdot}CH_2{\cdot}CH_2{\cdot}X$, where X and Y are nitrogen or a double bond, is deduced.

Thus the presence of two groupings has been established by n.m.d.r. $CH_3CH{:}C{\cdot}CH_2X$ and $CH_2{:}C{\cdot}CH(Y){\cdot}CH_2{\cdot}CH_2{\cdot}X$. The case where $X = N_b$ and $Y = C{:}C$ is present in structure (VII; $R = CH_2{:}$) but not in (VIII; $R = CH_2{:}$). Thus (VII; $R = CH_2{:}$) is established for N_b-methyldihydroapparicine and since it has been established that N_b is attached to C-6 in the alkaloid itself, it follows that (IX) represents apparicine. It should be noted that the alternative possibility (X) for N_b-methyldihydroapparicine which would be consistent with the nuclear magnetic resonance analysis above, is incompatible with the identification of pyridine-3,4-dicarboxylic acid from dehydrogenation and oxidation of apparicine.

The 100 Mc./sec. n.m.r. spectrum (Figure 2) of apparicine (IX) itself, though more complicated than that (Figure 3) of N_b-methyldihydroapparicine (VII) is completely consonant with the structure (IX) deduced above for the base. Aside from the main features already discussed, the spectrum contains a broad singlet (3·9 δ) which can now be assigned to the doubly allylic proton on C-2. This signal overlies the low field line of the A part (centered at 3·75 δ) of an AB pattern. The B part occurs in the region 2·8 to 3·6 δ. These two protons are the ones on the carbon atom C-7, situated between the basic nitrogen and the ethylidene double bond. The ethylidene methyl group was shown, by decoupling, to be interacting with both the proton at C-2 and one of the protons (that corresponding to the A part of the AB pattern discussed above) on C-7, as well as with the olefinic proton at C-9. The signals of two more protons occur in the region 2·8—3·6 δ and represent the two hydrogens adjacent to N_b on C-4. Finally the multiplet between 1·8 and 2·35 δ corresponds to the signals of the two saturated hydrogen atoms, on C-3.



The structure of apparicine is seen to be a novel variant on that of uleine (I). Each base contains the same carbon skeleton, but in apparicine, the *N*-methyl group of uleine has become the methylene bridge, C-6, located between the indole β-position and N_b. The biogenetic precursor suggested by Wenkert [10] for uleine is (XI). An unexceptional prototropic shift could transform (XI) into (XII), which with subsequent electrophilic attack

[10] E. Wenkert, *J. Amer. Chem. Soc.*, 1962, **84**, 98.

on the indole ring would lead to the species (XIII). Expulsion of the sugar residue, as suggested by Wenkert in the uleine biogenesis and transformation of the formyl group into exocyclic methylene and the acetyl group into ethylidene would then lead directly to the structure (IX) established for appariciné. Further credence is lent to such a formal scheme by the recent findings of Barton,[11] that *N*-methyl groups are the biogenetic precursors of methylene bridges in the berberine alkaloids and also by the isolation [1] of uleine congeners with a CH_2OH group instead of the exocyclic methylene substituent.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Mass spectra were determined by Drs. H. Budzikiewicz and M. Ohashi; infrared, ultraviolet spectra, and rotations were measured by Mrs. Aguilar and microanalyses were carried out by Messrs. E. Meier and J. Consul. The n.m.r. spectra and decoupling experiments were performed on a Varian HR 100 spectrometer. Samples were dissolved in deuteriochloroform containing tetramethyl silane as internal reference. The 100 Mc./sec. spectra were compared with 60 Mc./sec. spectra from a Varian A60 to aid in distinguishing coupling patterns from chemical shifts.

Apparicine (IX).—The alkaloid, crystallised from acetone, had m. p. 192—194° with a phase change to fine needles at *ca.* 160°, $[\alpha]_D^{27}$ ±177° (*c* 2·16 in $CHCl_3$) (Found: C, 81·48; H, 7·50; N, 10·71; *C*-Me, 5·21%; *M*, by mass spectrometry, 264. Calc. for $C_{18}H_{20}N_2$: C, 81·78; H, 7·63; N, 10·60; 1 *C*-Me, 5·68%; *M*, 264), $\lambda_{max.}$ 303 mμ (log ε 4·67), $\lambda_{min.}$ 265 mμ (log ε 3·79), $\lambda_{infl.}$ 230, 312 mμ (log ε 4·70, 4·61) in EtOH, $\lambda_{max.}$ in $CHCl_3$ 2·90 μ (NH str.).

Apparicine methiodide was obtained as a white amorphous powder by reaction of the base with methyl iodide in benzene at room temperature and subsequent removal of solvent.

N_b-*Methyl*-6-*methoxydihydroapparicine* (IV; R = OCH_3).—To (−)-apparicine methiodide (60 mg.) in methanol (5 c.c.) was added a solution of sodium (200 mg.) in methanol (5 c.c.). After 0·25 hr. at room temperature the solution was poured into a mixture of water (100 c.c.) and ether (50 c.c.). The ether layer was separated, dried, and evaporated. The residue (40 mg.) was crystallised from ether to give N_b-*methyl*-6-*methoxydihydroapparicine*, m. p. 154—157° $[\alpha]_D^{28}$ −145° (*c* 0·29 in $CHCl_3$) (Found: C, 77·01; H, 8·38%; *M*, by mass spectrometry, 310. Calc. for $C_{20}H_{26}N_2O$: C, 77·38; H, 8·44; *M*, 310), $\lambda_{max.}$ 223, 292 mμ (log ε 3·56, 3·16), $\lambda_{min.}$ 258 mμ (log ε 2·98) in EtOH, $\lambda_{max.}$ in $CHCl_3$ 2·89 μ (NH str.), 1·63 (3 proton doublet J = 7 c./sec. CH_3CH:), 2·21 (3 proton singlet CH_3N), 3·38 (3 proton singlet CH_3O), 4·61 (2 proton singlet β-indole·CH_2·O), 5·20 to 5·70 (3 protons CH_3CH: and CH_2:), 7·0 to 7·8 (4 protons *H*Ar), and 8·1 to 8·4 δ (1 proton N*H*).

N_b-*Methyldihydroapparicine* (VII; R = CH_2).—(−)-Apparicine methiodide (130 mg.) was treated with lithium aluminium hydride (100 mg.) in refluxing tetrahydrofuran (30 c.c.) for 0·25 hr. Excess of reagent was decomposed with water, the slurry filtered after dilution with ether (50 c.c.), and the dried filtrate evaporated. The residue (80 mg.) was crystallised from ether to give N_b-*methyldihydroapparicine*, m. p. 100—102°, $[\alpha]_D^{27}$ −140 (*c* 0·3 in $CHCl_3$) (Found: C, 81·45; H, 8·51%; *M*, by mass spectometry, 280. Calc. for $C_{19}H_{24}N_2$: C, 81·38; H, 8·63%; *M*, 280), $\lambda_{max.}$ 227, 295 mμ (log ε 3·60, 3·15), $\lambda_{min.}$ 264 mμ (log ε 2·80) in EtOH.

N_b-*Methyltetrahydroapparicine* (VII; R = H,CH_3).—Apparicine (100 mg.) was hydrogenated at room temperature over Adams catalyst in ethanol until one mol. had been absorbed. The residue after removal of solvent and the catalyst was purified by several precipitations from hexane. This amorphous material, which was homogeneous on thin-layer chromatography, was converted into its methiodide and reacted with lithium aluminium hydride in the manner described above, to give N_b-*methyltetrahydroapparicine* (VII; R = H, CH_3) (6 mg.), m. p. 128—135° (Found: *M*, by mass spectrometry, 282. Calc. for $C_{19}H_{26}N_2$: *M*, 282), $\lambda_{max.}$ 226, 284, 292 mμ (log ε 4·59, 3·91, 3·84) in EtOH, 1·08 (3 proton doublet, J = 7 c./sec. CH_3CH:), 1·38 (3 proton doublet, J = 7 c./sec. CH_3CH), 2·19 (3 proton singlet CH_3N), 2·30 (3 proton singlet CH_3Ar), 3·4 (1 proton multiplet ArCH·CH_3), 5·15 (1 proton quartet, J = 7 c./sec., CH_3CH:), 6·9 to 7·5 (4 protons *H*Ar), and 7·6 δ (1 proton N*H*), *m/e* 282 (5%), 158 (84), 143 (16), 124 (100), 122 (15).

Dehydrogenations.—In a typical experiment, a mixture of apparicine (20 mg.) and 30% Pd–charcoal (70 mg.) was heated in a sealed tube at 250° for 10 min. The resulting material was extracted with methanol and the residue after removal of solvent oxidised at 100° with

[11] D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *Proc. Chem. Soc.*, 1963, 267.

aqueous potassium permanganate. The excess of oxidant was destroyed with formaldehyde and the resulting solution passed through a column of Amberlite IRC-50. The eluate was concentrated and examined by paper chromatography for pyridine acids by the method of Kuffner.[7] In three different solvent systems the unknown acids moved with the same R_F values as pyridine-3,4-dicarboxylic acid and pyridine-3-carboxylic acid.

In one experiment the total pyridine acid fraction was esterified with diazomethane and the resulting ester examined mass spectrometrically. The spectrum corresponded to that of dimethyl pyridine-3,4-dicarboxylate and was different from the spectra of other pyridine diesters, *m/e* 195 (4%), 194 (5), 164 (100), 136 (14), 105 (23), 93 (23), and 78 (88).

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