Phytochemistry, Vol. 25, No. 6, pp. 1461–1468, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

ABEREAMINES, WATER-SOLUBLE SEED ALKALOIDS FROM *HUNTERIA UMBELLATA*

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(*Revised received* 18 *November* 1985)

Key Word Index—*Hunteria umbellata*; Apocynaceae; seed; alkaloids; isomeric delactonized 14-isopropyl-dihydroxydeoxyisocorymines.

Abstract—The isolation and characterization by spectroscopic methods of four isomeric 14-isopropyl-dihydroxydeoxyisocorymines with the lactone bridge open are described. These are water-soluble alkaloids named abereamines from the seeds of *Hunteria umbellata*.

INTRODUCTION

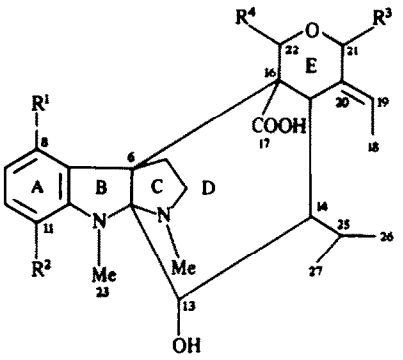
The plant *Hunteria umbellata* has been extensively investigated. All its parts contain alkaloids [1, 2]. From the leaves, erinine, ericinine and eripine have been isolated [3, 4] and umbellamine, a dimeric indole–indoline alkaloid from the root bark [5]. The structure of the latter compound was established by a detailed study of its mass spectrum and those of its *O*-acetyl, *O*-methyl and derived diol derivatives, coupled with thermolysis and a study of its ^{1}H NMR spectrum. Bevan *et al.* on the other hand isolated corymine, acetylcorymine and isocorymine from the seeds [6]. The UV spectra for the aforementioned indole alkaloids were similar and exhibited a marked shift in acid medium characteristic of the Ar–N–C–N system [7]. A comparison between the mass spectral fragmentation patterns of erinine and erinicine and derivatives and those of corymine, acetylcorymine and isocorymine combined with other spectroscopic data led to a revised structure for isocorymine [6].

Previous work associated no local medicinal use with the seeds [6]. The present investigation has, however, uncovered important local ethnomedical uses of the aqueous or alcoholic extracts of the seeds as a cure for piles, yaws, diabetes, stomach ulcer, etc. in some parts of Western Nigeria. Attention was therefore directed for the first time, to the water-soluble alkaloids of the seeds. The four alkaloids isolated possessed the isocorymine skeleton, but all differed from it by possessing a C-14 isopropyl substituent, an additional hydroxyl group inducing positional isomerism with the lactone bridge linking C-13 to C-16 open.

RESULTS AND DISCUSSION

NMR and mass spectral data showed that compounds **1**–**4** were isomeric, having a relative molar mass of 458 and a molecular formula $C_{25}H_{34}N_2O_6$.

The four indole alkaloids were precipitated from aqueous acid solution by means of Mayer's reagent and the dry complex decomposed by prolonged standing in 95% ethanol. Removal of solvent then afforded a mixture which was initially separated by flash chromatography on deactivated alumina followed finally by silica gel prep. TLC purification. Compounds **1**–**4** were distinguished by their R_f values (H_2O–*n*-BuOH–NH_4OH, 2:15:1) of 0.1, 0.3, 0.6 and 0.9, respectively.



1 $R^1 = R^2 = OH$; $R^3 = R^4 = H_2$
2 $R^1 = H$; $R^2 = R^4 = OH$; $R^3 = H_2$
3 $R^1 = R^2 = H$; $R^3 = R^4 = OH$
4 $R^1 = H$; $R^2 = R^3 = OH$; $R^4 = H_2$

The mass spectrometric fragmentation pattern of each of the isomers **1**–**4** was paralleled by that proposed for the revised structure for isocorymine [6] (Scheme 1). Ions **8A** *m/z* 325, **13** *m/z* 227 and **10C** *m/z* 199 are also characteristic of isocorymine. The prominence of fragment **11**, *m/z* 327, in the four probe spectra was significant. This was 16 mu greater than the corresponding isocorymine ion and indicated the presence of an additional hydroxyl group in **1**–**4** consistent wth the existence of rings A, B (ruptured) C and the tetrahydropyran ring E. Fragments **8**–**10**, consisting of essentially a benzo-azaindole ring system corroborated the evidence. These fragments originated from a rearrangement involving the indole–pyrrolidine skeleton [6]. In addition an ion at *m/z* 43 in each spectrum was

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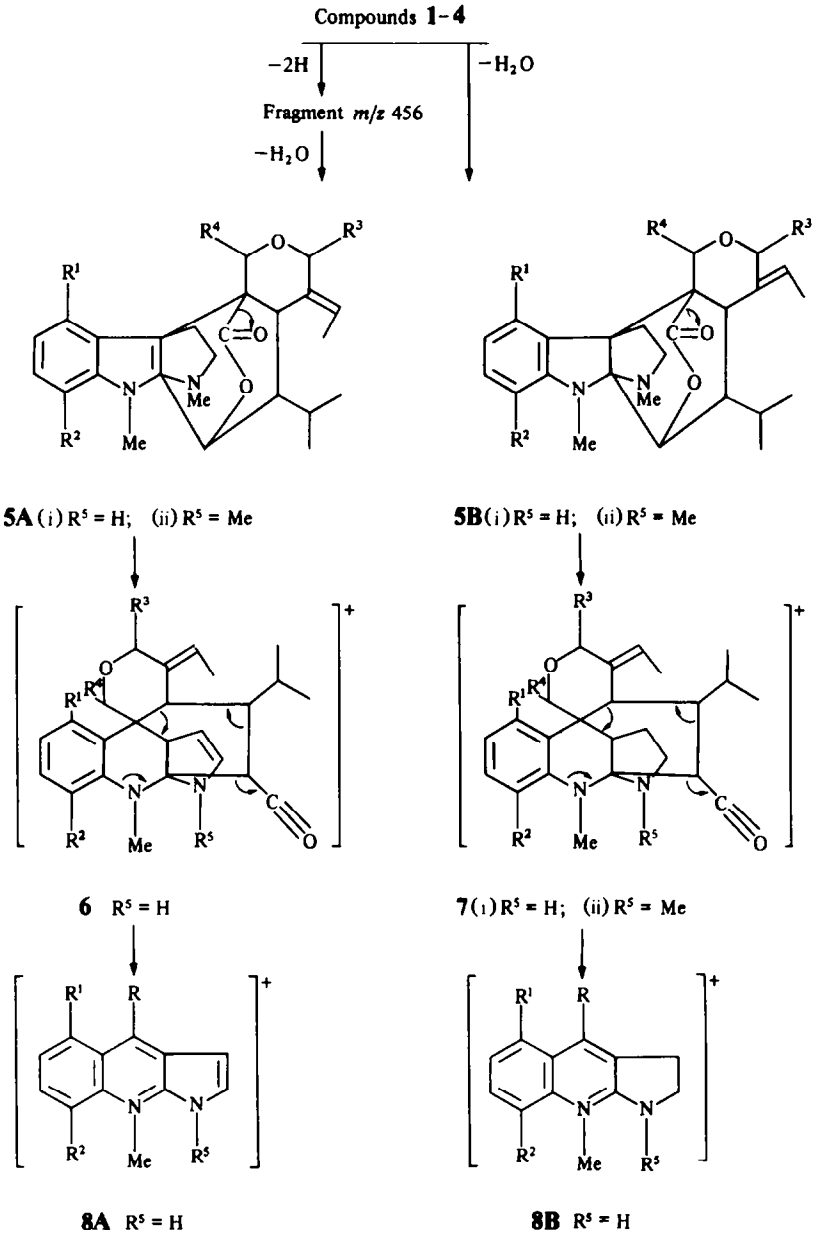
characteristic but was not accompanied by a corresponding *m/z* 415 $[M-43]^+$. However, the appearance of a minor fragment **14**, *m/z* 155, in all spectra could possibly arise as a result of bond ruptures between C-2 and C-13, C-15 and C-20, C-6 and C-16, and C-16 and C-22. The fragment contained the isopropyl subtituent.

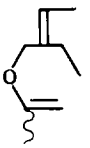
The IR spectra showed characteristic absorptions at *ca* ν_{max}^{KBr} cm^{-1}: 3340 (OH), 1710 (COOH), 1610 (aromatic ring), 1450 (–C–O–C–) and 1383 (isopropyl residue). There was no lactone absorption in any of the IR spectra. Each isomer therefore possessed the deoxyisocorymine skeleton with a collapse of its lactone bridge reappearing as C-13 hydroxyl and C-16 carboxy groups. In addition, the skeleton contains an isopropyl substituent at C-14 and two hydroxyl groups which were responsible for the isomerism.

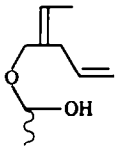
The ^1H NMR data (80 MHz in Me_2CO-d_6) for compounds **1–4** are summarized in Table 1. Compound **1** had a signal at δ7.8 as a singlet for two aromatic hydrogens. This invoked an equivalence on H-9 and H-10. The isomerism inducing dihydroxy groups were accordingly assigned as OH-8 and OH-11, respectively. An IR absorption at 807 cm^{-1} was characteristic of two adjacent aromatic hydrogens. In its mass spectrum the appearance of a minor ion **15a**, *m/z* 110, derived from a non-hydroxy-bearing tetrahydropyran ring coupled with the existence of ions **12a**, *m/z* 279 and **12c**, *m/z* 285 was in favour of the assigned structure.

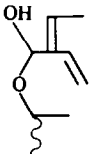
Compound **2** exhibited ^1H NMR signals at *ca* δ7.97 indicating three aromatic protons and a hydroxyl hydrogen absorbing at δ3.90 suggested the presence of a phenolic hydroxyl group. The trisubstituted pattern of the

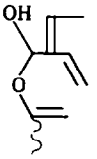


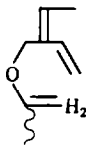
Scheme 1. Fragmentation of abereamines **1–4** to pyrrol- and dihydropyrroloquinolinium ions **5–10**. In Schemes 1–3, the letters a, b, c and d refer to fragments originating from compounds **1**, **2**, **3** and **4** respectively.

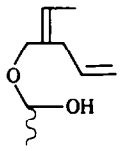
8A (a) R =  $R^1 = R^2 = OH$; *m/z* 325

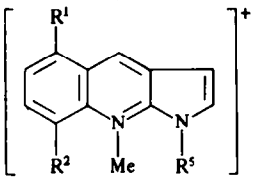
8A (b) R =  $R^1 = H$; $R^2 = OH$; *m/z* 325

8A (c) R =  $R^1 = R^2 = H$; *m/z* 325

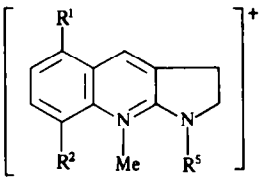
8A (d) R =  $R^1 = H$; $R^2 = OH$; *m/z* 325

8B (a) R =  $R^1 = R^2 = OH$; *m/z* 327

8B (b) R =  $R^1 = H$; $R^2 = OH$; *m/z* 327

8A → 

9 $R^1 = R^2 = R^5 = H$; *m/z* 183

8B (R^5 = H or Me) → 

10(a) $R^1 = R^2 = OH$; $R^5 = H$; *m/z* 217
(b) $R^1 = H$; $R^2 = OH$; $R^5 = Me$, *m/z* 215
(c) $R^1 = R^2 = H$; $R^5 = Me$; *m/z* 199
(di) = 9, *m/z* 183
(dii) = 10c; *m/z* 199
(diii) $R^1 = H$; $R^2 = OH$; $R^5 = Me$; *m/z* 215 obs. 217

Scheme 1 (*Continued*)

Table 1. ^{1}H NMR data* for abereamines

	1	2	3	4
H-8	—	7.97 *m*	7.23 *dd* (7.1, 2.0)	7.87 *m*
OH-8	5.49 *br*	—	—	—
H-9	7.83	7.97	7.08 *dd* (7.0, 2.1)	7.87
H-10	7.83	7.97	6.89 *dd* (7.2, 2.0)	7.87
H-11	—	—	7.31 *dd* (7.0, 22)	—
OH-11	5.49 *br*	3.90 *br*	—	5.53 *br*
—	5.49 *br* OH-13	3.80 *br* OH-13 and OH-22	5.01 *br* OH-13, OH-21 and OH-22	3.70 *br* OH-13 and OH-21
OH-17	—	—	9.9 *s*	—
H-18	2.06 *d* (7.01)	2.01 *d* (6.95)	2.09 *d* (6.95)	2.03 *d* (7.30)
H-19	5.49 *q* (2.20)	4.86 *q* (2.11)	5.09 *q* (2.29)	5.53 *q* (1.99)
H-21 ($-OCH_2C=$)	2.84 *s*	3.52 *s*	—	—
H-21 OCH(OH)C=	—	—	3.89 *s*	3.56 *s*
H-22 OCH_2C	2.84 *s*	—	—	3.31 *s*
H-22 OCH(OH)C	—	3.54 *s*	3.81 *br s*	—
H-23	2.09 *d* (1.90)	2.09 *d* (1.91)	2.73 *s*	2.12 *d* (1.90)
H-24	2.12 *s*	2.12 *s*	3.01 *s*	2.73 *s*
H-26	1.28 *d* (0.20)	0.89 *d* (0.03)	1.14 *d* (0.28)	0.92 *d* (0.06)
H-27	1.28 *d* (0.01)	0.89 *d* (0.03)	1.14 *d* (0.28)	0.92 *d* (0.06)

*Chemical shifts are in δ-values and ^{1}H NMR spectra were run in Me_2CO-d_6 at 80 MHz. Compound 3 was also run in Me_2CO-d_6 but at 400 MHz. *J* values in Hz are the numbers in parentheses.

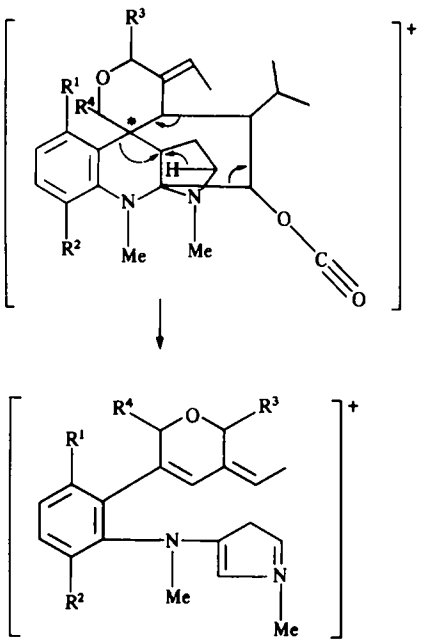
benzene nucleus here was further substantiated by a strong IR absorption at 750 cm^{-1}. However, the hydroxyl group could be sited at C-18 or C-11. The N-1 methyl signal was a doublet (J = 1.21 Hz), probably due to forced splitting of N-1 methyl by the hydroxyl proton. The placement of the aromatic hydroxyl group at C-11 was therefore most plausible. A ^{1}H NMR signal at δ3.52 (2H, *s*) for the $-OCH_2-C=$ system was comparable to that of isocorymine [6]. The other isomerism-inducing hydroxyl group was therefore located at C-22.

Four distinct adjacent aromatic hydrogens absorbing at δ6.89, 7.08, 7.23 and 7.31 were readily identified in the ^{1}H NMR of compound **3**. This observation was consistent with its IR absorption at 736 cm^{-1} for an *ortho* disubstituted benzene. The two hydroxyl groups were therefore placed at C-21 and C-22, respectively. Fragment **11c**, *m/z* 327 was prominent in its mass spectrum and served as further support for the structure assigned.

In compound **4**, three adjacent aromatic hydrogens appeared at *ca* δ7.87 in its ^{1}H NMR spectrum and had a similar splitting pattern to those of the aromatic protons in compound **2**. One of the hydroxyl groups was sited at C-11 unambiguously. IR absorption at 752 cm^{-1} also supported the presence of a 1,2,3-trisubstituted benzene. A consideration of the structures already proposed for compounds **1–3** left C-21 as the only site for the second hydroxyl group in compound **4**. ^{1}H NMR signals at δ3.31 (2H, *s*) for the group $-OCH_2-C-$ in **4** was in fact comparable to a value of δ2.84 (2H, *s*) for a similar group in **1**.

The two roving hydroxyl groups could occupy positions C-4 and C-5, respectively, in the pyrrolidine ring C. This was not the case since ions **10c**, *m/z* 199 (Scheme 1) and **13**, *m/z* 227 (Scheme 3) were prominent in each of the mass spectra examined. Each of the ions contained ring C with C-4 or C-5 carrying two hydrogen atoms.

As mentioned earlier, the fragmentation of each of the alkaloids followed a pattern similar to that described for isocorymine [3, 6] (Schemes 1–3). Apparently **1–4** lactonized to **5** with or without loss of two hydrogen atoms from ring C and by the loss of water from an interaction of the carboxyl and the C-13 hydroxyl groups. This might be followed by loss or retention of the N-3 methyl. Lactone **5** then rearranged; this involved an enlargement of ring B and resulted in **6** and **7**. To release strain, fragmentation initiated by newly formed bonds between either C-2 and



11

11(a) $R^1 = R^2 = OH$; $R^3 = R^4 = H_2$; *m/z* 327
(b) $R^1 = H$; $R^2 = R^4 = OH$; $R^3 = H_2$; *m/z* 327
(c) $R^1 = R^2 = H$; $R^3 = R^4 = OH$; *m/z* 327
(d) $R^1 = H$; $R^2 = R^3 = OH$; $R^4 = H_2$; *m/z* 327

Scheme 2. An alternative fragmentation pattern of rearranged intermediate **7** of abereamines **1–4** to 2-dihydropyranylanilinium ions **II**.

C-16 or C-14 and C-16, α to the carbonyl converted **6** to **8A** and **7** to **8B** or **11**. The first route led to fragmentation of **6** to **8A** and **7** to **8B** while the alternative bond fission in **7** gave fragment **11** (Scheme 2). By loss of either residue, **8A** and **8B** fragmented further to the ions **9** and **10**, respectively. The low mass ether residues were identified as ions **15** (Scheme 3). The conversion of **7** to **11** by the alternative route (Scheme 2) involved an initial loss of CO_2 followed by cleavage of bonds between C-14 and C-15, C-6 and C* and a new one C-2 and C-16 in fragment **11**.

The probe spectra contained the following metastables: *m/z* 456, 329, 327, 325, 279, 254, 199, 149 and 127. Ion *m/z* 329 was characteristic of compounds **1** and **3** 327 of **2** and **4** 325 of **3** and **4** 279 of **1** and 254 and 127 of **2**, **3** and **4**. Ions 199 and 456 were characteristic of the four compounds. Although fragment *m/z* 227 was not a metastable, it was prominent in all four spectra (Scheme 3). The origin of this and *m/z* 254 in the spectra of **2**, **3** and **4** and *m/z* 279 in **1** could be explained by the assigned structures **13**, **12b** and **12a**, respectively. Ion *m/z* 254 might result from loss of one hydrogen atom from **12b**. By loss of ethylene **12b** transformed readily into the aldehydic ion **13**, which by loss of its formyl group afforded the basic ion **10c**. Ion **12c** was a precursor of **12b** characteristic of compound **3**.

The four water-soluble alkaloids, abereamines, were delactonized 14-isopropyl-dihydroxydeoxyisocorymines and 14-isopropyl-hydroxyisocorymines and have been assigned sturctures **1–4**. Compound **1** was identified as delactonized 8,11-dihydroxy-14-isopropyl-22-deoxyisocorymine, **2** as delactonized 11-hydroxy-14-isopropylisocorymine, **3** as delactonized 21-hydroxy-14-isopropylisocorymine and **4** as delactonized 11,21-dihydroxy-14-isopropyl-22-deoxyisocorymine.

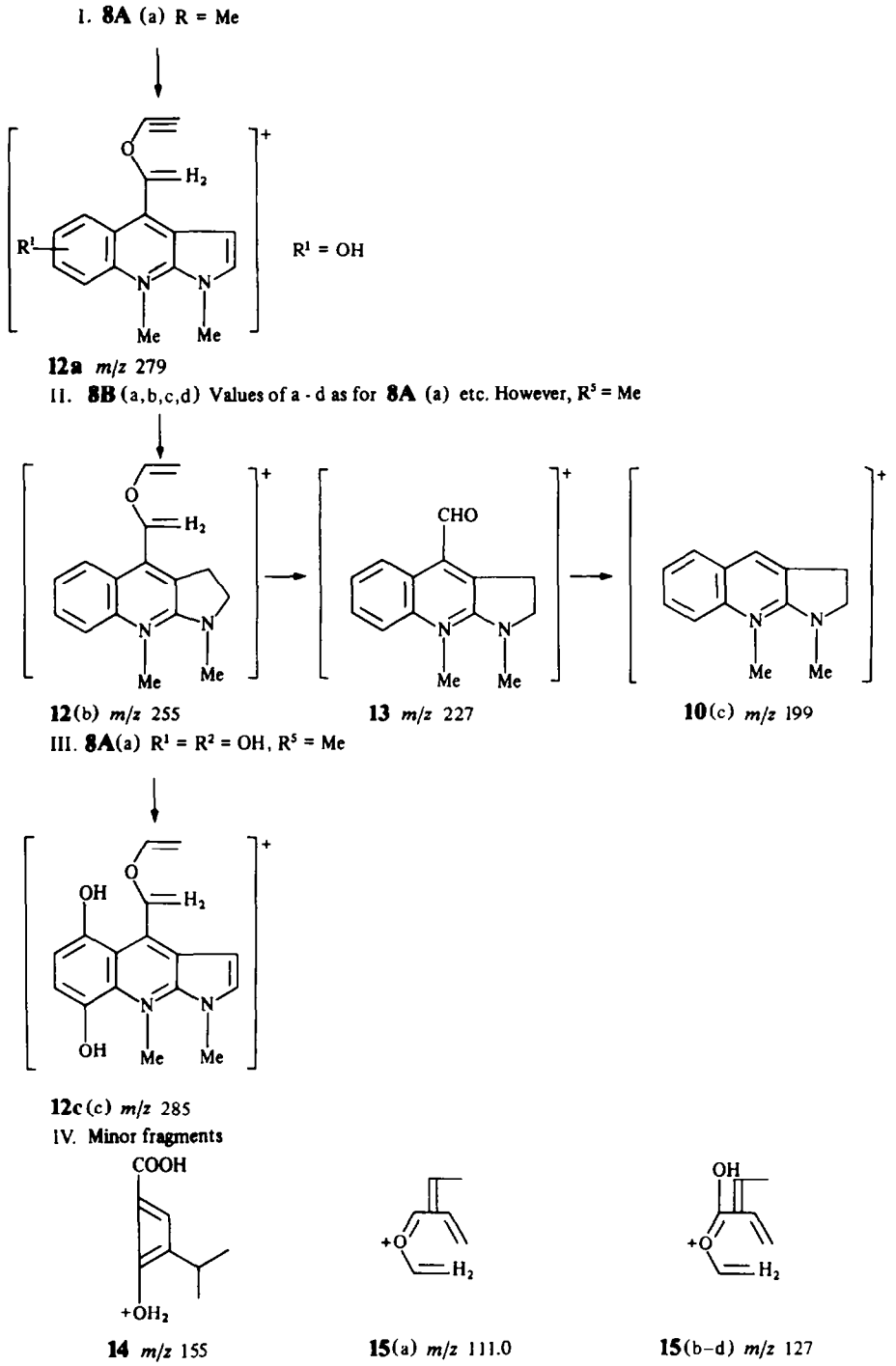
It is now appropriate to discuss briefly the possible biosynthetic pathway to the new alkaloids. Noteworthy are the additional three carbon atoms the skeleton possesses in addition to that of any other alkaloid isolated to date from same source. Mono N-methylation of both amino functions in tryptamine by methionine followed by Schiff base reaction between the amine and secologanin, both derivable from the acetate, gives an adduct which cyclizes to **16** (Scheme 4). Structure **16** readily hydroxylates at C-6 to give **17**. Loss of the hydroxyl group immediately effects ring closure via a C-16 electrophilic attack on C-6. This is followed by C-22 hydroxylation which may or may not be followed by deoxygenation resulting in **18**. Structure **18** rearranges to **19** but reverts to **20** with the bond between C-15 and C-14 retained and the other bond linked to C-14 ruptured. The C-15 carbomethylene ion eliminates a proton to give an exocyclic double bond compelling **20** to assume the more stable structure **21** in which the olefinic bonds at C-15 and C-20 are both exocyclic and conjugated. The zwitterions **20** and **21** must be very transient. Structure **21** is, however, long-lived enough to allow incorporation of the 3-hydroxybutanoic acid cation (via butanoic acid-3-phosphate derivable from acetoacetic acid by H^+, NADPH reduction followed by ATP phosphorylation) into the C-15 double bond terminal carbon atom. By a 1,2 proton shift from C-15 to C-14, structure **22** is formed via C-13 and C-14 bond linkage and the decarboxylation of the isobutanoic acid residue attached to C-14. Molecular rearrangement is completed by a rupture of the bond between C-13 and N-3, the rupture being initiated by the ring 'C' electrophilic quarternary nitrogen. This finally contracts ring 'C' from six to five, the whole system resulting in a carbocation which permits ready C-13 hydroxylation. Hydrolysis converts the C-16 ester to the acid and the C-21 glycoside to the hydroxyl group.

EXPERIMENTAL

Mps are uncorr. ^1H NMR spectra were determined in Me_2CO-d_6 with TMS as int. standard. IR spectra were taken in KBr pellets and EIMS at 70 eV.

Extraction. Pulverized seeds of *H. umbellata* (K. Sohum) Hall. F. (750 g) were continuously extracted with EtOH at 100° over a period of 3 days. Solvent was removed from the particle-free soln by distillation first at ordinary pres. and then at red. pres. affording a dark brown residue (39.35 g). The procedure, after two repetitions on the spent wood material, afforded an additional residue (4.10 g). The EtOH extract was labelled M_1.

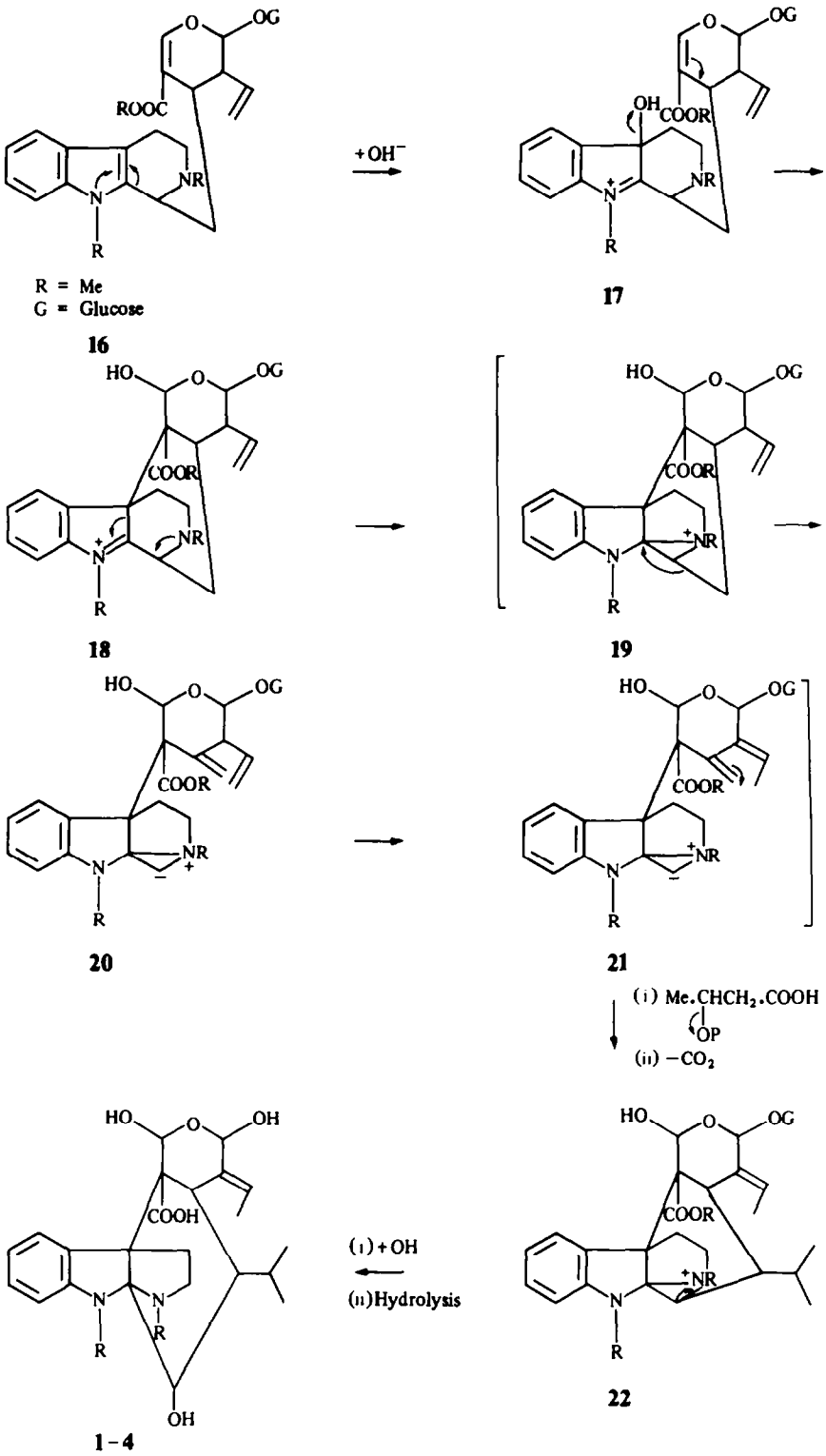
Separation. The brown residue M_1 was repeatedly extracted with small portions of 5% aq. HCl until a test sample gave negative tests with Dragendorff's and Mayer's reagents. The combined acid soln was carefully basified with 5% Na_2CO_3 soln and the mixture extracted repeatedly with small portions of $CHCl_3$ until the last extract was almost colourless. The combined $CHCl_3$ extract afforded after removal of solvent a mixture of non-H_2O-soluble alkaloids (20.1 g) labelled M_2. This was not investigated further. The alkaline aq. filtrate from M_2 was re-acidified with 5% aq. HCl and the resulting soln treated with a slight excess of Mayer's reagent. The ppt was filtered and air-



Scheme 3. Origin of metastables and some important minor fragments in alkaloids **1**–**4**.

dried, yielding a mixture M_3 containing the H_2O-soluble alkaloids as a brownish-yellow Mayer's complex (5.1 g). M_3 was dissolved in 95% EtOH (500 ml) and the soln allowed to stand at room temp. for 1 week after which the complex had decomposed. Evaporation of the filtered EtOH mixture left a residue M_4 (2.9 g) containing the H_2O-soluble alkaloids. A preliminary separation of M_4 on deactivated alumina (basic) gave compound **4** by elution with Et_2O, **3** with EtOAc, **2** with MeOH–EtOAc (1:1) and **1** with MeOH–EtOAc (3:1).

Further purification was achieved by prep. TLC (silica gel) in H_2O–*n*-BuOH–NH_4OH (2:15:1). Each component was then recovered by extraction into MeOH. Removal of solvent afforded compound **1**, R_f 0.1, as a yellow solid (110 mg), mp 199° (dec.); IR ν_{max}^{KBr} cm^{-1}: 3477, 1717, 1621, 1607, 1450, 1383 and 807. MS



Scheme 4. A possible biosynthetic route to the abereamines 1–4.

m/z (rel. int.): 458 $[M]^+$ ($C_{25}H_{34}N_2O_6$), 456 (17), 329 (3), 327 (2), 325 (1), 279 (2), 227 (1), 217 (1), 199 (2), 155 (1), 111 (9). See Table 1 for ^{1}H NMR data.

Compound 2, R_f 0.3, was a red solid (500 mg), mp 230° (dec.); IR ν_{max}^{KBr} cm^{-1}: 3547, 1725, 1636, 1616, 1449, 1383 and 750. MS *m/z* (rel. int.): 458 $[M]^+$ ($C_{25}H_{34}N_2O_6$) 456 (83), 329 (15), 327 (13), 325 (6), 254 (9), 227 (5), 217.10 (2), 199 (7), 155 (3), 127 (35), 111 (2). See Table 1 for ^{1}H NMR data. Compound 3, R_f 0.6, was a

Table 2. ^{13}C NMR data (δ-values) for compound 3

Carbon No.	δ_C
2	32.664
4	14.436
5	2.751
6	30.755
7	178.231
8	202.605
9	203.110
10	203.391
11	207.884
12	179.186
13	77.368
14	28.845
15	29.800
16	31.709
17	180.141
18	14.637
19	144.784
20	121.900
21	111.510
22	78.884
23	35.46
24	34.068
25	27.892
26	26.936
27	29.800

Chemical shifts in δ-values were measured in Me_2CO-d_6 at 80 MHz.

brown solid (120 mg), mp 280° (dec.); IR ν_{max}^{KBr} cm^{-1}: 3675, 1717, 1625, 1607, 1458, 1383 and 736. MS m/z (rel. int.): 458 $[M]^+$ ($C_{25}H_{34}N_2O_6$) 456 (100), 329 (12), 327 (9), 325 (4), 254 (7), 227 (5), 199 (7), 155 (1), 127 (42), 111 (2). The ^1H NMR spectrum was determined at 400 MHz (Table 1) and the ^{13}C NMR at 80 MHz (Table 2).

Compound 4, R_f 0.9 was also a red solid (100 mg), mp 260–262°. IR ν_{max}^{KBr} cm^{-1}: 3548, 1720, 1625, 1615, 1451 and 1383. MS m/z (rel. int.): 458 $[M]^+$ ($C_{25}H_{34}N_2O_6$), 456 (100), 329 (19), 327 (15), 325 (6), 279 (1), 254 (13), 227 (5), 199 (8), 155 (3), 149 (10), 127 (54), 111 (8). See Table 1 for ^1H NMR data.

Acknowledgements—The support of the Central Research Committee University of Lagos is acknowledged for making a grant available for this work. Acknowledged also is the effort of Prof. T. A. Emokpae, the Chairman of the Faculty of Science Research Committee at the time the grant was awarded. We thank Mr. John Adegoke for his unflinching technical assistance throughout the course of the programme. Finally we thank Prof. V. Snieckus, University of Waterloo, Ontario, Canada and the Southwestern Ontario High-Field NMR-Facility for the 400 MHz NMR.

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