Tabernaelegantinines C and D, Two New Bisindole Alkaloids containing a Cyano Group from *Tabernaemontana elegans* Stapf. Part 2.[1,†]

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Tabernaelegantinines C and D, isolated from the root bark of *Tabernaemontana elegans*, have been shown by spectroscopic data and chemical correlation to be ' dimer ' alkaloids composed of a dregamine unit and an isovoacangine unit connected through a linkage involving C(3) of the former and C(12') or C(10') of the latter, respectively. The isovoacangine unit of both compounds contains an extra cyano-group at C(3') and the configuration at this centre is (*S*).

We have recently described [1] the structure of six new bisindole alkaloids, tabernaelegantines A—D (1)—(4) and tabernaelegantinines A and B (5) and (6), isolated from the roots of *Tabernaemontana elegans* Stapf (Apocynaceae). We now report the structure of two new closely related bisindole alkaloids, tabernaelegantinines C and D (7) and (8).

High-resolution mass spectrometry established the formula $C_{44}H_{53}N_5O_5$ for tabernaelegantinine C (Found: M^+, 731.402 9. Calc.: M, 731.404 2). Physical data and u.v., i.r., and n.m.r. spectra are reported in Tables 1—3. The spectral properties of tabernaelegantinine C closely resemble those of tabernaelegantines A and C [1] and clearly demonstrate that in (7) the mode of linkage of the two ' halves ' of dihydrovobasine and isovoacangine involves the C(3) and C(12') positions, respectively.

TABLE 1

Physical constants and i.r. and u.v. data

Compound	M.p. (°C) (solvent)	C.d. λ/nm (Δε) (MeOH)	$\lambda_{max.}$/nm (log ε) (95% EtOH)	$\nu_{max.}$/cm^{-1} (KBr)	R_F *
(7)	177°	203	224	3 380	0.39
	(MeOH)	(+12.6)	(4.76)	(NH)	
		224	285	2 230	
		(−94.3)	(4.19)	(CN)	
		237	293	1 730	
		(+77.5)	(4.15)	(ester)	
(8)	259°	203	226	3 410	0.34
	(decomp.)	(+14.8)	(4.74)	(NH)	
	(MeOH)	223	287	2 230	
		(−50.4)	(4.18)	(CN)	
		237	295	1 725	
		(+51.9)	(4.16)	(ester)	
(9)	229°	204	225	3 450	0.37
	(decomp.)	(+16.7)	(4.76)	(OH)	
	(MeOH–H_2O)	225	286	3 370	
		(−96.3)	(4.18)	(NH)	
		337	294	1 720	
		(+76.2)	(4.15)	(ester)	

* On silica gel G (Merck F 254); eluant n-hexane–acetone (1 : 1).

Furthermore, a sharp absorption at 2 230 cm^{-1} in the i.r. spectrum and the difference between molecular formula of (7) and those of tabernaelegantines A and C suggest the presence of an additional CN group.

The mass spectrum (electron impact; 70 eV, 160°) of (7) is the same in character as that of tabernaelegantines but shows certain shifts. In addition to the molecular ion at *m/e* 731 (50%) and the intermolecular methyl transfer ion [2] at *m/e* 745 ($M^{+\cdot}$ + Me − H, 19), there are small peaks at *m/e* 718 (745 − 27, 11), 704 ($M^{+\cdot}$ − 27, 7), 700 ($M^{+\cdot}$ − $MeNH_2$, 7), 687 (745 − 58, 7), 673 ($M^{+\cdot}$ − 58, 8) and diagnostically valuable intense ions at *m/e* 549 ($C_{37}H_{37}N_4O_3$, $M^{+\cdot}$ − 182; *m** 412.3, 77), 536 ($C_{33}H_{36}N_4O_3$, $M^{+\cdot}$ − 195, *m** 393.0, 59), and 418 ($C_{25}H_{28}N_3O_3$, 10). The fragments at *m/e* 700, 673, 549, 536, and 418, which correspond to ions *a'–e'* which arise by the same path as ions *a–e* of tabernaelegantines, are shifted by +25 a.m.u. with respect to the latter, showing that the CN group is present in the isovoacangine ' half '. Ions *c'—e'* eject HCN to give ions at *m/e* 522 ($C_{33}H_{36}N_3O_3$, 42%), 509 ($C_{32}H_{35}N_3O_3$, 42), and 393 ($C_{24}H_{27}N_2O_3$, 5) as supported by the corresponding metastable transitions *m** 496.3, 483.4, and 365.7, respectively. In the low-mass region, fragments with *m/e* 196 (*f*, 26%), 182 (*g*, 100), and 124 (*i*, 19), characteristic of the dihydrovobasine residue, are apparent as well as the fragments with *m/e* 136 (*h*, 14%) and 122 (*j*, 27) all typical of the aliphatic part of the isovoacangine ' half '. Unlike the high-mass fragments, these last two ions do not seem to contain the CN group.

TABLE 2

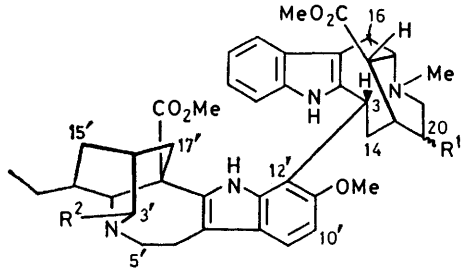
^{1}H N.m.r. spectra (δ) *

	Compound (7)	(8)	(9)
NH	7.64	7.74	7.74
	7.54	7.55	7.58
ArH	7.24 [a]	7.02—6.85 [c] (5 H)	7.4—6.9 [c] (5 H)
	7.2—6.9 [c] (4 H)	6.79 [b]	6.88 [a]
	6.85 [a]		
C-3-H	5.24 [c]	5.05 [c]	5.24 [c]
ArOMe	3.96	3.93 [d]	4.00
CO_2Me	3.70	3.69	3.70
NMe	2.62	2.64	2.64
C(17)O_2Me	2.53	2.55	2.55
CH_2*Me*	0.93 [e]	0.93 [e]	0.92 [e]
CH_2*Me*	0.84 [e]	0.89 [e]	0.84 [e]

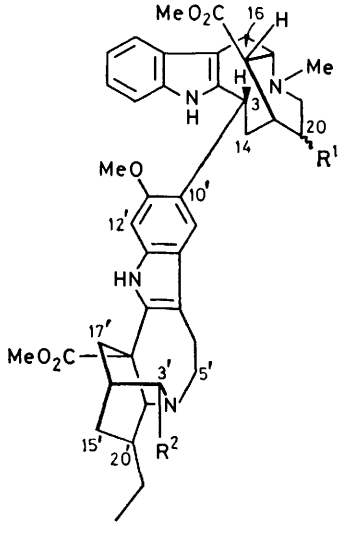
* 100 MHz; $CDCl_3$ as solvent; Me_4Si as internal standard.
[a] 1 H, d, *J* 9 Hz. [b] 1 H, s. [c] m. [d] br, $W_{1/2}$ 6 Hz. [e] *J* 6 Hz.

† This work was presented at the 11th International Symposium on Chemistry of Natural Products, Golden Sands, Bulgaria, 1978.

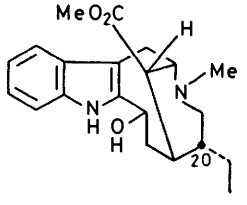
The presence of a CN group is unusual in indole alkaloids and additional evidence was sought to verify its placement as well as the configuration at C(20) in the dihydrovobasine moiety. This was forthcoming when the ^{13}C n.m.r. spectrum was examined (see Table 3).



(1) R^1 = β-Et, R^2 = H
(3) R^1 = α-Et, R^2 = H
(5) R^1 = β-Et, R^2 = CH_2COCH_3
(7) R^1 = α-Et, R^2 = CN
(9) R^1 = α-Et, R^2 = OH



(2) R^1 = β-Et, R^2 = H
(4) R^1 = α-Et, R^2 = H
(6) R^1 = β-Et, R^2 = CH_2COCH_3
(8) R^1 = α-Et, R^2 = CN



(10)

TABLE 3

^{13}C N.m.r. data (δ) *

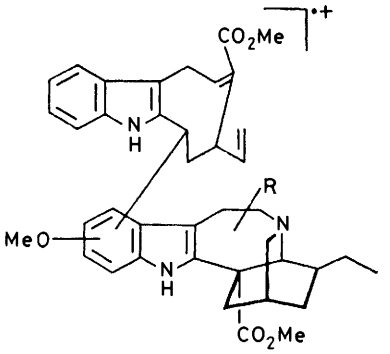
Carbon	Compound (7)	(8)	(9)
2	136.1	135.2	136.0
3	35.1	37.1	35.1
5	59.1	59.4	59.1
6	19.5	19.5	19.3
7	108.5	110.0	108.5
8	129.4	130.2	129.4
9	118.1	118.2	117.9
10	119.4	118.9	119.4
11	122.1	121.6	122.0
12	110.2	111.1	110.1
13	136.2	136.9	136.0
14	29.2	31.9	29.1
15	33.0	33.1	33.0
16	49.9	49.8	49.6
18	11.4	11.5	11.4
19	23.5	23.6	23.6
20	43.8	44.0	43.8
21	49.5	49.8	49.5
*C*O$_2$Me	171.8	172.1	172.0
CO$_2$*Me*	49.9	49.9	49.9
N*Me*	42.4	42.5	42.4
2′	135.2	134.3	135.8
3′	53.1	53.5	95.6
5′	51.6	52.0	52.3
6′	21.4	21.6	21.7
7′	109.8	110.0	109.8
8′	123.8	122.3	123.7
9′	117.0	117.7	116.9
10′	105.4	128.5	105.2
11′	152.2	154.0	151.8
12′	115.1	93.1	115.0
13′	138.0	134.5	135.1
14′	31.2	31.4	29.7
15′	28.4	28.6	24.6
16′′	53.7	54.3	53.7
17′	33.7	35.6	33.6
18′	11.4	11.5	11.6
19′	26.5	26.6	26.5
20′	38.0	38.3	37.6
21′	55.7	56.0	55.4
*C*O$_2$Me	173.4	174.8	174.0
CO$_2$*Me*	52.5	52.9	52.3
ArO*Me*	56.7	56.0	56.7
*C*N	120.0	120.4	

* Fourier-transform instrument operating at 25.2 MHz; $CDCl_3$ as solvent; Me_4Si as internal standard.

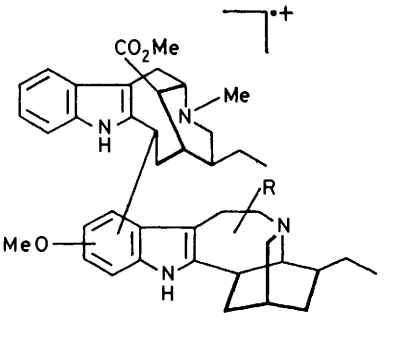
Most of the carbon resonances are readily assigned using standard chemical shift theory and by comparison with the previous assignment of the spectra of tabernaelegantines A and C, it is possible to identify the resonances of the 19,20-dihydrovobasine 'half'. In particular, the low-field resonance for C(16) in (7) (δ_C 49.9) is identical with that of tabernaelegantines C and D (δ_C 49.9 and 49.8),[1] and thus the stereochemistry at C(20) is certainly (*S*) as in dregaminol (10), *i.e.* the ethyl group is α-oriented.[3] For the isovoacangine moiety, the resonances of the aromatic carbons show good agreement with the proposed placement of the methoxy-group in tabernaelegantine C as well as good correspondence with some aliphatic carbon resonances, *i.e.* C(6′), C(16′), C(18′), C(19′), and C(20′). It is noteworthy that one of the aminomethylene carbon signals, C(3′) or C(5′), in the range δ 50—53 is missing and replaced by a methine signal, the C(14′) doublet resonance has a downfield shift (4.1 p.p.m.), whilst both C(21′) and C(15′) show upfield shifts (1.9 and 3.5 p.p.m., respectively). The

singlet resonance for CN at δ 120.0 is in excellent agreement with the literature data.[4]

The attachment of CN to C(3′) is expected on chemical-shift grounds. In fact, the major chemical-shift perturbations resulting from the introduction of a cyano-group occur at the α- (1—2 p.p.m. downfield), β- (2—3 p.p.m. downfield), and γ-positions (3—4 p.p.m. upfield) with respect to the analogous carbons in the unsubstituted skeleton.[5] Based on this notion, C(3′) is shifted from δ 51.3 in (3) to 53.1 in (7) due to direct substitution, C(5′) from 53.0 to 51.6 p.p.m. because of its γ-position. Moreover, C(2′) moves to δ 31.2 in (7) from 27.1 in (3), whilst C(6′) is unaffected. If the cyano-group is attached to C(3′) it must be oriented toward C(15′), *i.e.* the C(3′) configuration must be (*S*), in order to explain the upfield shift of this carbon due to a γ-steric interaction.



a R = H
a′ R = CN



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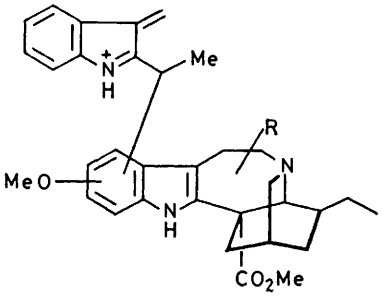
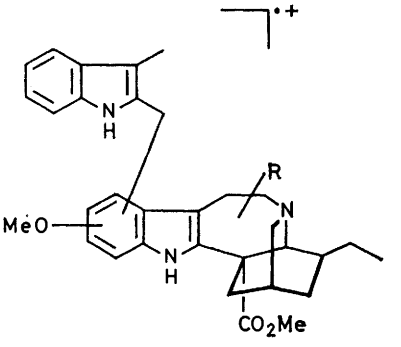
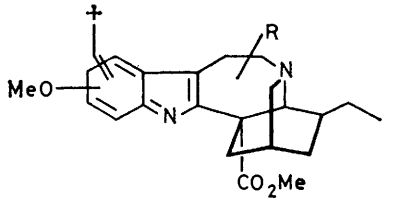
b′ R = CN



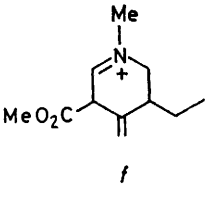
c R = H
c′ R = CN



d R = H
d′ R = CN



e R = H
e′ R = H



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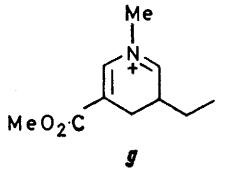

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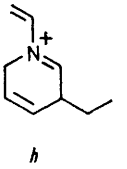

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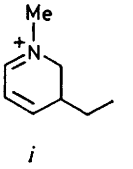

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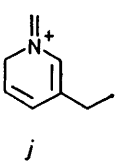
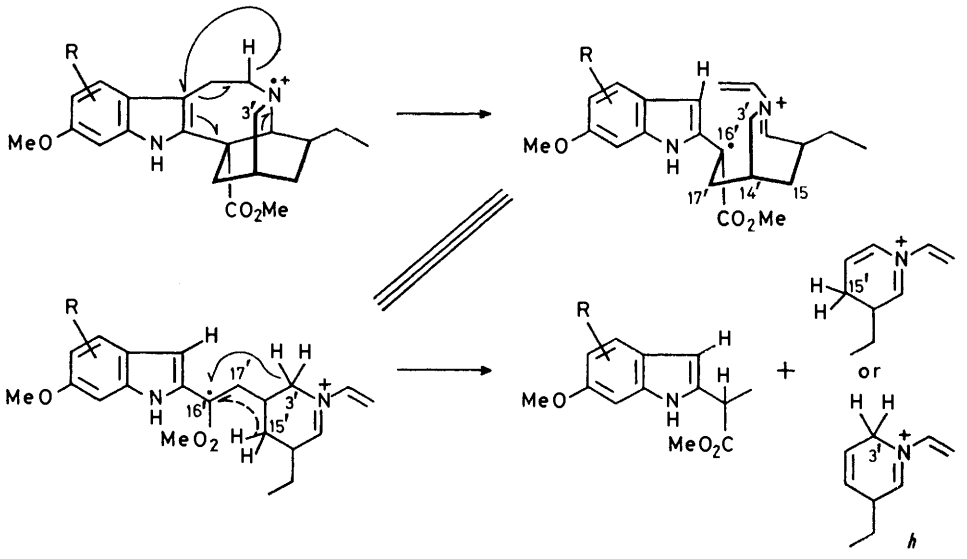


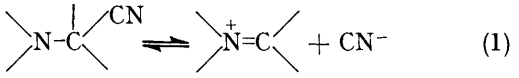
j

the CN group at C(3′) is unequivocally substantiated by its reductive removal with $NaBH_4$ which follows the well known behaviour of α-aminonitriles.[6] The conversion of (7) into tabernaelegantine C (3) is accomplished in high yield only in the presence of Co^{II}, Ni^{II}, and Cu^{II} salts. The presence of these salts is crucial to the success of the reaction since in their absence only traces of (3) are observed. It is plausible to suggest that these transition-metal ions promote the fragmentation of α-aminonitriles by scavenging the cyanide ions [equations



SCHEME 1

(1) and (2)]. Interception of the resulting iminium cation by $NaBH_4$ through hydride transfer to the carbon leads to removal of the cyano-group.[6,7] Metal(II)-



(1)

$$M^{III}L_n + CN^- \rightleftharpoons M^{III}L_{n-m}(CN)_m \qquad (2)$$

assisted reductive removal of the CN group with $NaB[^2H_4]$ gives monodeuteriotabernaelegantine C [3′-$^2H_1$]-(3), whose ^{13}C n.m.r. spectrum is essentially identical with that of the protio-isomer, except for the expected dramatic decrease of the intensity of the methylene resonance at δ 51.3, due to quadrupole broadening, spin–spin coupling, and decreased nuclear Overhauser enhancement.

The mass spectrum of [3′-$^2H_1$]-(3) at 160° displays in the molecular ion region, peaks at *m/e* 707 [53.7% relative to peak at *m/e* 182(100)] and 706(12.6) indicating 81% deuterium incorporation. According to the proposed fragmentation pattern,[8] ions at *m/e* 196 (*f*) and 182 (*g*) are not shifted while peaks at *m/e* 136 (*h*), 124 (*i*), and 122 (*j*) are partially shifted by +1 a.m.u. The intensity ratio of *m/e* 137 to *m/e* 136 is 1 : 0.75, whereas for the pair of peaks of *m/e* 125—124 and 123—122 the ratio is 0.82 : 1 and 1 : 0.92, respectively. The deuterium-labelling experiment demonstrates that the genesis of the *m/e* 136 fragment involves hydrogen transfer from C(3′) and C(15′) to the C(16′) radical site of the eliminated moiety (Scheme 1). In an earlier paper we reported that the *m/e* 124 peak arises from the dihydrovobasine ' half ', according to the fragmentation pattern of vobasine alkaloids.[9] However the presence of the *m/e* 125 ion does not exclude the possibility that the *m/e* 124 ion, at least in tabernaelegantine C (3), arises, in part, by a different path, *i.e.* from the isovoacangine ' half ' through the disproportionation of the radical-ion (*l*) (Scheme 2).

According to the proposed structure, (7) is converted quantitatively by acid-catalysed hydrolysis (2N-HCl in refluxing MeOH–H_2O 1 : 1) into (9). Its ^{13}C n.m.r. spectrum (Table 3, see Tables 1 and 2 for other physical and spectral data) displays a doublet resonance at δ 95.6 due to C(3′), whereas C(15′) is shifted 7.2 p.p.m. upfield and C(14′) 2.6 p.p.m. downfield compared with (3). This trend is consistent with the (*R*)-configuration at C(3′), *i.e.* with the hydroxy-group *syn*-oriented with respect to the ethyl chain at C(20′).

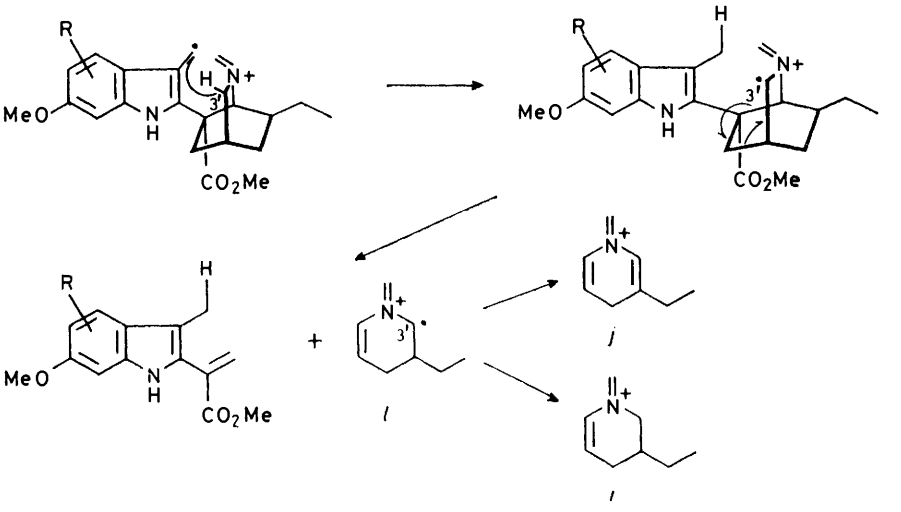
Tabernaelegantinine D (8) contains the same ' halves ' but with the modification that C(3) of the dregamine moiety is attached to C(10′) of the isovoacangine ' half ' and not to C(12′) as in (7). The extra cyano-group is, as in the previous case, at C(3′) with the (*S*)-configuration at this centre, and the reductive removal of the CN group with $NaBH_4$–M^{II} leads to tabernaelegantine D(4).[1] There are some noteworthy differences in the chemical and spectroscopic behaviour of (7) and (8). When (8) is hydrogenated (room temperature; 1 atm; AcOEt) in the presence of 10% Pd–C, tabernaelegantine D (4) is the sole product (20% after 48 h), while (7) is found to be unchanged under the same conditions.

Unlike (7), tabernaelegantinine D (8) shows a mass spectrum highly dependent upon the temperature. At

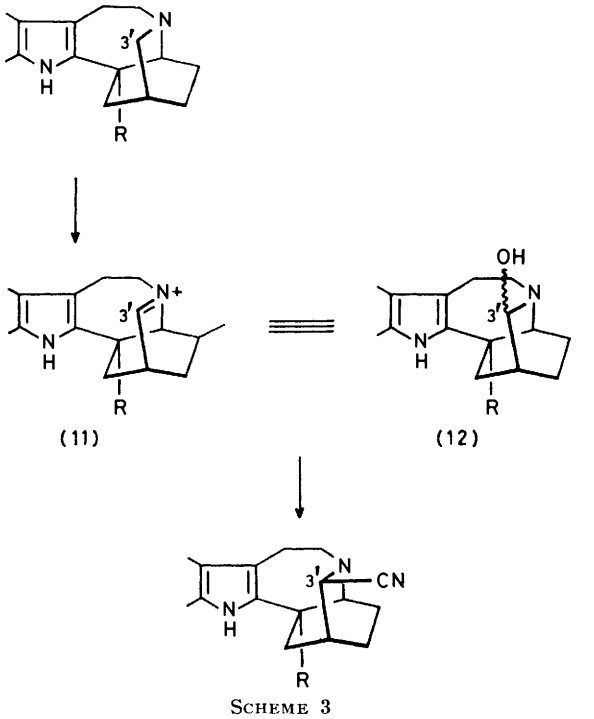
160° it displays, beside the molecular ion at *m/e* 731 (27.7%) and the methyl transfer ion at *m/e* 745 ($M^{+\cdot}$ + Me — H, 9), intense peaks at *m/e* 718 (704 + Me — H, 10.2%), 704 (731 — 27, 40.7), 549 (55.5), 536 (64.8), 522 (88.8), 509 (100), 195 (55.5), and 182 (100). In addition, other prominent features in the high mass region of the spectrum are the large abundance of ions at *m/e* 720 (10.2%), 706 (38.3), 524 (50.0), and 511 (51.8) arising from the presence of molecules in which formal substitution of the CN group by hydrogen has taken place. On raising the temperature to 180°, the intensity of the *m/e* 731 and 745 ions decreases (8.1 and 8.75% relative to the base peak), while that of ions at *m/e* 704, 706 and 718, 720 (20.9, 21.6, and 18.4, 18.4) increases. At 200° there is a decrease in the intensity of the pairs of ions at *m/e* 731, 745, and 704, 706 [1.7, 5.5, and 7.8, 7.9 relative to the ion at *m/e* 182 (100)] while the methyl transfer ions at *m/e* 718 and 720 (28.5 and 25.3) predominate.* The intensity ratio of the pairs of ions at *m/e* 704, 706, and 718, 720 appears to be independent of the vaporisation temperature. Moreover, introduction of [$^2H_2$]O into the mass spectrometer does not shift the *m/e* 706 and 720 ions, thus ruling out the participation of water adsorbed in the spectrometer in their formation. All these facts can be rationalized in terms of some degree of thermal decomposition in the inlet system resulting in the loss of CN·, followed by intermolecular hydrogen transfer (disproportionation) to afford dehydrogenated species [formal elimination of HCN from starting material; *m/e* 704 ($M^{+\cdot}$) and related ions] and hydrogenated species [formal substitution of CN by H in the starting molecules; *m/e* 706 ($M^{+\cdot}$) and related ions].

A possible explanation of the different catalytic and thermal behaviour of (7) and (8) can be obtained from inspection of molecular models. Because rotation about the C(3)–C(10′) bond is sterically less inhibited that around the C(3)–C(12′) bond, the N–C(3′)–CN system of the isovoacangine unit in (8) appears much less crowded by the dregamine unit than in (7) and consequently more suitable to proton addition.

The most likely biosynthetic route to molecules such as tabernaelegantinines C and D can be summarized in Scheme 3 involving the intermediacy of the iminium salt (11) or the equivalent hydroxy-derivative (12) which subsequently undergoes nucleophilic addition or displacement by cyanide anion.†



SCHEME 2



SCHEME 3

EXPERIMENTAL

Conversion of Tabernaelegantinine C (7) *into Tabernaelagantine C* (3).—(a) *By cobalt*(II)*-assisted reductive removal of*

* The increase of intensity of methyl transfer ions with temperature agrees well with the presence of a C(3)–C(10′) bond and has been previously observed.[2]

† We rule out totally the introduction of a cyanide ion during the isolation and separation procedures.

CN. To a stirred solution of tabernaelegantinine C(7) (72 mg) in methanol (50 ml), cobalt(II) chloride (100 mg) was added under nitrogen and the mixture was stirred for 2 h at room temperature. The solution was then cooled at 0° and $NaBH_4$ (72 mg) in methanol–water (99 : 1; 30 ml) was added. After stirring at 40° for 2 h, the mixture was evaporated *in vacuo* and the residue taken up in hot dichloromethane. The resulting solution was dried, filtered on alumina, and concentrated *in vacuo* to afford pure tabernaelegantine C (3) (65 mg), confirmed by t.l.c. and spectral comparisons.

(b) *By catalytic hydrogenation.* A solution of tabernaelagantinine C (50 mg) in ethyl acetate (50 ml) was hydrogenated for 48 h at room temperature and 1 atm. in the presence of 10% Pd–C (5 mg). The filtered solution was concentrated and the residue chromatographed on two silica gel preparative plates, developing with n-hexane–acetone (1 : 1). The band at R_F 0.35 gave tabernaelegantine C (9.5 mg).

Synthesis of [3′-$^2H_1$]*Tabernaelegantine C.*—From tabernaelagantinine C (144 mg) and NaB[$^2H_4$] (99% ^2H, 150 mg) in methanol as above, [3′-$^2H_1$]tabernaelagantine C (130 mg) was obtained, *m/e* (160°) 708 (26%), 707 (M^+, 53.7), 706 (12.6), 705 (7.4), 525 (100), 524 (12), 512 (61), 511 (7), 394 (9), 353.5 (M^{2+}, 5), 196 (18), 182 (67), 137 (30), 136 (22.5), 125 (15), 123 (18), and 122 (16.5).

Conversion of Tabernaelegantinine D (8) *into Tabernaelagantine D* (4).—(a) *By cobalt*(II)*-assisted reductive removal of* CN. By the above procedure, (8) was reduced quantitatively to (4).

(b) *By catalytic hydrogenation.* Under the above condition, (8) was recovered completely unchanged after 48 h.

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