Isolation and Identification of Three New Phenolic Furoquinoline Alkaloids from *Teclea verdoorniana* Exell & Mendonça (Rutaceae)

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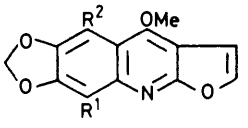
Separation of the stem bark extracts of *Teclea verdoorniana* Exell & Mendonça (Rutaceae) yielded three new phenolic furoquinoline alkaloids, 8-hydroxy-5-(3-methylbut-2-enyl)-6,7-methylenedioxydictamnine (tecleaverdoornine) (1), 8-hydroxy-5-(3-hydroxy-3-methylbutyl)-6,7-methylenedioxydictamnine (tecleaverdine) (7), and 8-hydroxy-6,7-methylenedioxy-dictamnine (tecleine) (8), along with known alkaloids flindersiamine and kokusaginine. The pentacyclic triterpene lupeol was also isolated in large quantities. The application of ethanolic hydrochloric acid as a selective 4-*O*-demethylating reagent for dictamnine-type alkaloids is discussed.

In continuation of our work on West African medicinal plants of the family Rutaceae, we have examined chemically the extracts of the stem bark of *Teclea verdoorniana* Exell & Mendonça [1] collected from Nkolbisong near Yaoundé, Cameroon. We now present a full account of our work on the isolation and characterisation of three new phenolic furoquinoline alkaloids, tecleaverdoornine (1), tecleaverdine (7), and tecleine (8), and the known alkaloids flindersiamine (2) and kokusaginine (14) from the combined hexane and chloroform extracts.[2]

T.l.c. showed the presence of at least ten compounds



(1) R^1 = OH, R^2 = CH_2·CH=CMe_2
(2) R^1 = OMe, R^2 = H
(3) R^1 = OAc, R^2 = CH_2·CH=CMe_2
(4) R^1 = OMe, R^2 = CH_2·CH=CMe_2
(5) R^1 = OH, R^2 = CH_2·CH_2·C(Cl)Me_2
(6) R^1 = OH, R^2 = CH_2·CH_2·C(OCOH)Me_2
(7) R^1 = OH, R^2 = CH_2·CH_2·C(OH)Me_2
(8) R^1 = OH, R^2 = H
(9) R^1 = OAc, R^2 = H

in the combined extracts. Separation and purification by column chromatography on silica gel afforded six compounds reported here in decreasing order of elution.

Lupeol,[3] the major component (1%), was identified by direct comparison with an authentic sample from *Fagara Xanthoxyloides* Lam.[4]

Tecleaverdoornine (1) (0.003% based on the weight of dried bark), $C_{18}H_{17}NO_5$, formed neither a hydrochloride nor a picrate. Colour tests with iron(III) chloride (green) and gallic acid in hot concentrated sulphuric acid (emerald green; Labat test [5]) together with u.v. spectral data (Table 1), indicated that (1) was a furoquinoline [6] with at least a phenolic hydroxy-group and a methylenedioxy-group. The u.v. spectrum was similar to that of flindersiamine (2). The presence of a phenolic hydroxy-group was further confirmed by an i.r. absorption at 3 410 cm^{-1} and by the formation of a monoacetate (3) (M^+ 369; $\nu_{max.}$ 1 768 cm^{-1}), and a monomethyl ether (4) (M^+ 341). The methyl ether and the acetate showed no hydroxy absorption in their i.r.

Table 1

U.v. spectral data for the furoquinolines and their derivatives

Alkaloid	Solvent	$\lambda_{max.}$/nm	$\epsilon_{max.}$/ dm^3 mol^{-1}
(1)	$CHCl_3$	248	28 000
		263	58 000
		330	10 000
		350	6 500
	EtOH + OH^-	275	(Qualitative)
(2)	EtOH	245	40 000
		253	53 000
		338	5 000
(3)	EtOH	249	34 600
		255	40 700
		314	8 500
		323	7 800
		343	3 300
(4)	EtOH	249	44 600
		257	63 000
		318	13 500
		332	9 000
(6)	$CHCl_3$	363	78 000
		330	18 000
		352	10 500
(7)	EtOH	259.5	74 800
		322—345	13 800
(8)	EtOH	254.8	76 000
		320	12 000
		325	12 000
		340	9 600
	EtOH + OH^-	270	63 300
		320	12 400
		327	11 800
(10)	$CHCl_3$	248	18 000
		367	39 000
		355	12 000

spectra. The presence of a methylenedioxy-group was confirmed by i.r. absorption at 1 475 and 923 cm^{-1} (ref. 7) and a two-proton singlet at δ 6.10 in the ^1H n.m.r. spectrum. The ^1H n.m.r. spectrum (Table 2) also showed signals for a methoxy-group at δ 4.35 (3 H, s), a phenolic hydroxy-group at δ 7.54 (1 H, exchanged with D_2O), and a 3,3-dimethylallyl (prenyl) group at δ 1.70 and 1.80 (singlet methyls), 3.80 (2 H, d, *J* 7.5 Hz, benzylic methylene), and 5.30 (1 H, t, *J* 7.5 Hz). No aromatic

TABLE 2

^{1}H Chemical shifts (δ; 60 MHz) of tecleaverdoornine, tecleaverdine, tecleine, and their derivatives [a]

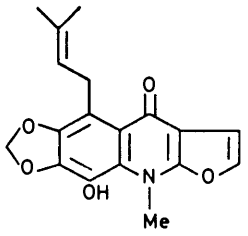
Compound	1-H (1H)	2-H (1H)	4-OMe (3H)	OCH_2O (2H)	–CH= (1H)	CH_2 (2H)	CMe (6H)	Others
(a) Derivatives with an unmodified 3,3-dimethlyallyl group								
(1)	7.55 (d) (*J* 2)	7.10 (d) (*J* 2)	4.35	6.10	5.30 (t) (*J* 7)	(3.80) (d) (*J* 7)	1.70 1.80	7.54 (8-OH)
(4)	7.55 (d) (*J* 2)	6.98 (d) (*J* 2)	4.34	6.02	5.31 (t) (*J* 7)	3.82 (d) (*J* 7)	1.70 1.80	4.20 (8-OMe)
(3)	7.55 (d) (*J* 2)	7.05 (d) (*J* 2)	4.35	6.12	5.30 (t) (*J* 7)	3.85 (d) (*J* 7)	1.70 1.80	2.50 (8-OAc)
(10)	7.20 (d) (*J* 2)	6.96 (d) (*J* 2)		6.05	5.50 (t) (*J* 7)	4.08 (crude d) (*J* 7)	1.69 1.81	4.06 [b] (8-OMe) 3.93 [b] (N-Me)
(b) Derivatives with a modified 3,3-dimethylallyl group: $XMe_2C{\cdot}CH_2{\cdot}CH_2Ar$					Me_2C	CH_2	CH_2	
(5)	7.50 (d) (*J* 2.5)	7.02 (d) (*J* 2.5)	4.38	6.05	1.63	2.00 (m)	3.33 (m)	7.48 (8-OH)
(6)	7.57 (d) (*J* 2.5)	7.08 (d) (*J* 2.5)	4.40	6.08	1.62	2.02 (m)	3.10 (m)	8.15 (formate H) 7.58 (8-OH)
(7)	7.54 (d) (*J* 2.5)	7.05 (d) (*J* 2.5)	4.38	6.10	1.34	1.65—1.85 (m) (3H)	3.20 (m)	7.55 (8-OH)
(8)	7.57 (d) (*J* 2.5)	7.18 (d) (*J* 2.5)	4.40	6.12				7.31 (H-5)
(9)	7.55 (d) (*J* 2)	7.05 (d) (*J* 2)		6.12				2.50 (8-OAc) 7.40 (H-5)
(2)	7.58 (*J* 2)	7.02 (d) (*J* 2)	4.38	6.08				4.27 (8-OMe) 7.26 (H-5)

[a] Tetramethylsilane as internal standard; unless otherwise indicated, all signals are singlets; coupling constants (*J*) are given in Hz. [b] Assignments tentative.

proton signal was observed. In addition, the low-field chemical shift (δ 4.35) of the only methoxy-group suggested that it must be at C-4.[8] This was also in accord with biogenetic considerations.[9] It therefore followed that tecleaverdoornine (1) is a tetrasubstituted dictamnine containing a hydroxy-group, a prenyl group, and a methylenedioxy-group on the aromatic homocyclic ring. The relative positions of these groups had, however, to be determined.

Position 5 was preferred for the prenyl group on spectroscopic evidence and biogenetic grounds. The abnormally low-field chemical shift (δ 3.80) of the benzylic methylene protons in (1), its acetate (3), and its methyl ether (4) (*cf.* δ 3.20—3.50 usually observed for these protons [10]) suggested deshielding by the neighbouring 4-methoxy-group, a suggestion supported by the fact that in the ^{1}H n.m.r. spectrum of the 4-quinolone, isotecleaverdoornine methyl ether (10), obtained by the action [11] of methyl iodide on tecleaverdoornine methyl ether (4), the benzylic protons absorbed at δ 4.10, demonstrating the stronger *peri*-effect of the carbonyl at C-4 in (10). Biogenetic considerations [9] also supported the 5-position for the prenyl group. Indeed, C-5 oxygenation is rare in the dictamnine-type alkaloids; [12] of the four furoquinolines positively shown to contain a modified *C*-prenyl group on the homocyclic ring, three of them, choisyine (19),[3] acronycidine,[3] and medicosmine,[3] bear this function at C-5.

An alternative structure (13) was eliminated on the following grounds. The co-occurrence of flindersiamine (2) with (1) in *T. verdoorniana* immediately suggested the flindersiamine oxygenation pattern for tecleaverdoornine (1). Also, in the ^{1}H n.m.r. spectrum of the methyl ether (4) the additional methoxy absorption at δ 4.20 compared well with the chemical shift (δ 4.27) observed [9] for 8-Me in (2). The mass spectra of (1) and (4) also gave fragments which were in agreement [13] with their structural relationship with (2). The fragment ion peaks for the methyl ether corresponding to $M - 1$, $M - 15$, $M - 29$, and $M - 71$ clearly pointed to 8-methoxylation [13] rather than 6-methoxylation. In addition, in the mass spectrum of compounds having a prenyl side-chain adjacent to a hydroxy-group [structure (13)], fragmentation occurs with loss of C_4H_6 (56 mass units).[14] A cyclic mechanism has been proposed [14] for the loss of this fragment. The mass spectral fragmentation of (1) with no significant peak at *m/z* 271 ($M - 56$) was inconsistent with structure (13). Structure (13) was finally ruled out when tecleaverdoornine (1) failed to give a chroman derivative (16) on treatment with concentrated hydrochloric acid in acetic acid. In the course of this reaction, however, a phenolic chloro-compound (5), $C_{18}H_{18}ClNO_5$, corresponding to the addition of the elements of hydrogen chloride to (1), was isolated. The structure of this compound was indicated by its i.r. absorption at 3 410 cm^{-1} (OH), by its ^{1}H n.m.r. spectrum [δ 3.33 (2 H, m, $ArCH_2{\cdot}CH_2$), 2.00 (2 H, m, $ArCH_2{\cdot}CH_2$), and 1.63 (6 H, s, CMe)],[15] and by the mass spectrum (M^+ 363). Another attempt at cyclising tecleaverdoornine (1) to the chroman derivative (16), this time with formic acid (98%) was also unsuccessful. Instead, a phenolic compound (6) $C_{19}H_{12}NO_7$, differing from (1) in the addition of the elements of formic acid, was obtained. It was identified as the formyloxy-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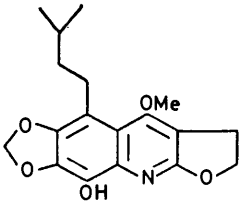
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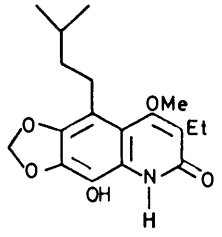
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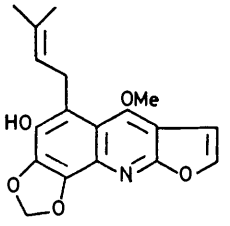
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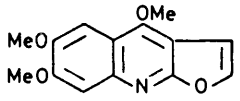
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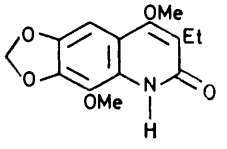
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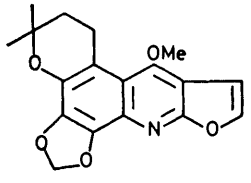
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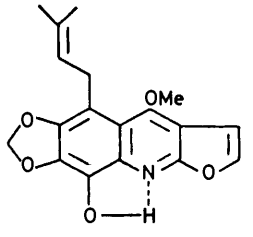
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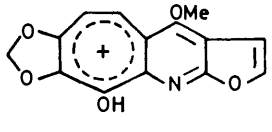
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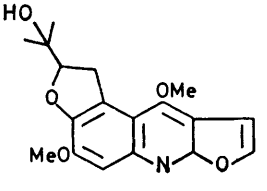
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compound (6) from its i.r. absorption at 1 695 cm^{-1} (formate CO) and its ^1H n.m.r. spectrum (Table 2), and by its ready hydrolysis to the hydroxy-derivative (7) (see later).

The above chemical and spectral data thus support structure (1) for tecleaverdoornine. Tecleaverdoornine (1) is the first tetrasubstituted dictamnine to be reported, and the only *C*-prenylfuroquinoline containing a fully aromatic homocyclic ring.

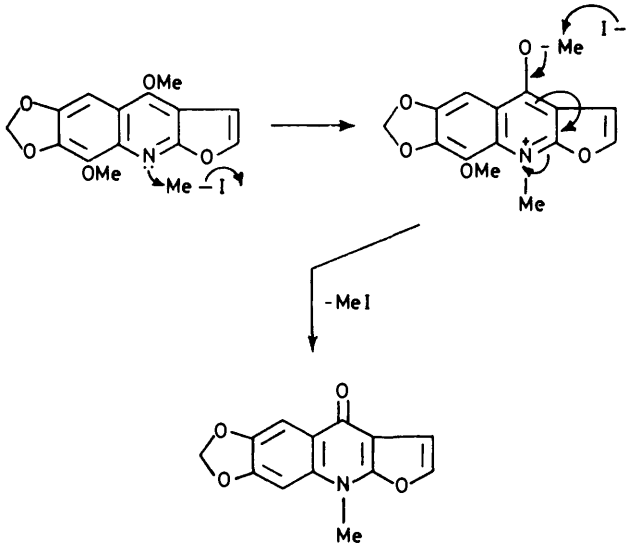
Some characteristic reactions of dictamnine-type alkaloids were performed on (1) to confirm its structure. Catalytic hydrogenation in dry ethyl acetate over palladium–carbon [12] afforded tetrahydrotecleaverdoornine (11) (M^+ 331), and reduction with platinum oxide (Adams' catalyst) in acetic acid caused simultaneous hydrogenation of the prenyl group and hydrogenolysis of the furan ring affording a hexahydro-derivative (12) [12] (M^+ 333). The structure of (12) was indicated by an i.r. absorption at 1 638 cm^{-1} (2-quinolone [16]) and by its u.v. spectral data (see Experimental section) which were typical of a 2-quinolone.[17] As expected, no acid shift was observed in the spectrum.

Tecleaverdoornine was recovered without demethylation after heating with ethanolic hydrochloric acid under conditions that led to the 4-*O*-demethylation of acronycidine [18] and skimmianine; [19] but failure to undergo demethylation at C-4 was not regarded as evidence for the alternative 4-hydroxy-8-methoxy-structure, as flindersiamine (2) could not be demethylated under the same conditions. We note that previous attempts by Johns *et al.*[20] to demethylate evolitrine and 7-demethylevolitrine and by Abe [21] to demethylate dictamnine also met with failure. No explanation was given for this apparently 'abnormal' behaviour of the 4-methoxy-group in these compounds. Nonetheless, our case and the previous ones discussed cast doubt on the general applicability of ethanolic hydrochloric acid as a selective 4-*O*-demethylating reagent for dictamnine-type alkaloids and call for a more detailed re-investigation of the mechanism of the reaction.

Compound (1) did not give isotecleaverdoornine when heated with methyl iodide in a sealed tube.[11] The failure of this reaction was attributed to hydrogen bonding between the 8-hydroxy-group and the electron pair on nitrogen (17). Since the isomerisation reaction is known [22] to take place according to the Scheme, this hydrogen bonding apparently makes the lone electron-pair unavailable for attack by the methyl iodide, and thus prevents the initial and crucial step of the reaction. The inability of (1) to form a hydrochloride and a picrate could similarly be explained by this H-bonding.

The second furoquinoline alkaloid from the extract was identified as tecleine (8).[23] This was first isolated in 1958 from *Teclea sudanica* by Paris and Stambouli, who recognised it as a hydroxymethylenedioxydictamnine,[23] but failed to complete the structural determination because of shortage of material. The identity of the isolate was established by direct comparison of its i.r. spectrum with that of the original tecleine. The colouration observed with iron(III) chloride (green) confirmed the presence of a phenolic hydroxy-group, responsible for an i.r. absorption at 3 440 cm^{-1}. A methylenedioxy-group was also indicated by a positive Labat test [5] and i.r. absorptions [7] at 2 780, 1 480, and 938 cm^{-1}. The u.v. spectrum (Table 1) exhibited striking similarity to

that reported [6] for flindersiamine (2), but underwent a bathochromic shift upon addition of base. The ^{1}H n.m.r. spectrum (Table 2) was consistent with structure (8). Of particular significance was the low-field chemical shift (δ 4.40) of the methoxy-group, which was assigned to C-4 in accord with data recorded for alkaloids of this



SCHEME Mechanism of MeI-induced isomerisation of dictamnine-type alkaloids

group.[9] Biogenetic considerations [10] coupled with the absence of significant $M - 1$ and $M - 29$ ion peaks in the mass spectrum of (8) eliminated the possibility of 8-methoxylation and further supported structure (8). Acetylation with acetic anhydride in acetic acid gave a monoacetate, $C_{15}H_{11}NO_6$, responsible for an enol acetate signal at δ 2.50 in the ^{1}H n.m.r. spectrum, while methylation with ethereal diazomethane yielded a methyl ether identical (i.r., u.v., n.m.r., and t.l.c.) with authentic flindersiamine (2).

The third and fourth alkaloids, eluted from the column together and further purified, were the known flindersiamine (2) [24] and kokusaginine (14).[25] Flindersiamine (2) was characterised by its m.p. (207—208°; lit.,[24] 206—207°) and those of its picrate and iso-derivative [25] (see Experimental section), and its spectroscopic data.[9] A hitherto unidentified derivative of (2), the 2-quinolone (15), was prepared by hydrogenolysis over Adams' catalyst in acetic acid and characterised. Kokusaginine was also identified by comparison of its physical and spectroscopic data with those previously reported.[9,25]

Tecleaverdine (7), the last alkaloid obtained, was isolated in very low yield (0.0025% with respect to dried bark), and gave the same colour reactions as (11). Recrystallisation from methanol gave colourless crystals, $C_{18}H_{19}NO_6$, confirmed by accurate exact mass determination. The u.v. spectrum was similar to that of tecleaverdoornine (1). Its n.m.r. spectrum (Table 2) showed that the prenyl side-chain was hydrated. This was confirmed by the mass spectrum, which exhibited prominent fragments at m/z 327 ($M - H_2O$), 312 ($M - H_2O - CH_3$), and 272 [base peak, presumably resulting from the cleavage of the hydrated prenyl group at the allylic position to form the stable tropylium-like cation (18)]. The relationship between tecleaverdoornine (1) and tecleaverdine (7) was finally established by oxymercuration–demercuration–hydration of the former to give the latter, which therefore must be (7). As earlier stated, (1) was also converted into (7) *via* the formyloxy-compound (6).

Grundon [26] has suggested a biosynthetic derivation of tecleaverdoornine (1) from 8-hydroxy-6,7-methylenedioxydictamnine which involves *C*-prenylation of the latter, the absence of a 6-hydroxy-group precluding oxidative cyclisation of the type which occurs in choisyine (19).[26] The characterisation of tecleine (8) in this investigation supports this suggestion. However, verification was not possible, as lack of sufficient (8) precluded a biomimetic synthesis of (1) based on *C*-prenylation. Attempted synthesis of tecleaverdoornine (1) by Friedel–Crafts reaction of flindersiamine (2) with prenyl bromide under a variety of conditions and catalysts was also unsuccessful, probably because of strong steric hindrance at C-5.

It is of interest that Cameroonian specimens of *T. verdoorniana* used in this study yielded only furoquinoline alkaloids, in complete contrast to specimens of this plant from Ghana which have been reported [27] to contain mostly acridones. This variation of chemical constituents of a plant species with the geographical location of the species is, however, not without precedent among West African Toddalioideae. An analogous situation has been reported with *Oricia sauveolens*; the Nigerian population contains only pyranoquinolines while plant material from Ghana furnished mostly acridone alkaloids.[27] Waterman [27] has suggested that such diversity in alkaloid production by a given plant species in different environments might be a sign of adaptation to different factors in the various habitats.

EXPERIMENTAL

U.v. spectra were recorded with a Perkin-Elmer 137 spectrophotometer and i.r. spectra with Perkin-Elmer 137 and 337 grating instruments. ^{1}H N.m.r. spectra were obtained with a Varian T60A or a Perkin-Elmer R12B spectrometer for solutions in deuteriochloroform unless stated otherwise. Mass spectra were determined with Hitachi–Perkin-Elmer RMU 6E and A.E.I. MS9 instruments.

Isolation of Constituents.—Large quantities of the stem bark were used in this investigation. In a typical procedure, the air-dried stem bark of *Teclea verdoorniana* (5.5 kg) collected from Nkolbisong in the Centre-South Province of Cameroon in October 1975 was powdered and Soxhlet-extracted successively with hexane (15 l) and chloroform (15 l) for 24 h. The two extracts, which were qualitatively the same (t.l.c.), were combined and concentrated under reduced pressure to yield a greenish paste (256 g). Part of this material (62.5 g) was chromatographed over silica gel

(1.5 kg) packed in hexane. Gradient elution was effected with hexane, hexane–ether mixtures, chloroform, and chloroform–methanol mixtures. A total of 212 fractions of about 250 ml each were collected and mixed on the basis of t.l.c. and ^{1}H n.m.r. data.

Lupeol.—Fractions 15—28 were mixed and rechromatographed over silica gel. Elution with hexane–diethyl ether (8 : 2) afforded lupeol (1% based on the weight of dried bark), which crystallised from dry ether as colourless needles, m.p. 212° (lit.,[3] 214—216°); acetate, m.p. 218° (lit.,[3] 220°). It was identical (i.r. and mixed m.p.) with an authentic sample.

Tecleaverdoornine (1).—The combined fractions 29—59, eluted with hexane–diethyl ether (8 : 2), on concentration left a green syrup. Treatment with methanol precipitated a solid, which was washed several times with methanol to leave crude *tecleaverdoornine* (1) (0.003% based on the weight of dried bark). Recrystallisation from acetone yielded long colourless needles, m.p. 191°, giving a green colouration with $FeCl_3$ and an emerald-green colour with a hot solution of gallic acid in concentrated sulphuric acid (Found: C, 65.95; H, 5.35; N, 4.1. $C_{18}H_{17}NO_5$ requires C, 66.05; H, 5.25; N, 4.3%); $\nu_{max.}$ (KBr) 3 410, 3 154, 3 130, 1 680, 1 610, 1 535, 1 475, 1 340, 1 270, 1 238, 1 190, 1 170, 1 148, 1 095, 1 066, 1 000, 968, 938, 923, 840, 800, 785, 772, 736, and 712 cm^{-1}; *m/z* 327 (M^+, 100%), 312 (57), 296 (9), 284 (12), 270 (6), 254 (7), 119 (4), 69 (5), and 41 (8); for u.v. and n.m.r. data see Tables 1 and 2 respectively.

Acetylation of tecleaverdoornine (1). Tecleaverdoornine (1) (100 mg) in pyridine (5 ml) was treated with acetic anhydride (5 ml) and left at room temperature for 5 h. The product was poured into cold distilled water (20 ml) and the resulting crystalline precipitate filtered off, washed with water and dried. Recrystallisation from methanol afforded colourless crystals of *tecleaverdoornine acetate* (3) (85 mg), m.p. 212° (Found: C, 65.05; H, 5.15; N, 3.55. $C_{20}H_{19}NO_6$ requires C, 65.05; H, 5.2; N, 3.8%); $\nu_{max.}$ (KBr) 3 160, 3 140, 1 768, 1 672, 1 618, 1 530, 1 465, and 925 cm^{-1}; *m/z* 369 (M^+, 12%), 328 (20), 327 (100), 312 (54), 296 (4), 284 (4), 272 (5), and 259 (28); for u.v. and ^{1}H n.m.r. data see Tables 1 and 2.

Methylation of tecleaverdoornine (1). Tecleaverdoornine (1) (250 mg) was dissolved in dry ethyl acetate, treated with an excess of diazomethane and left overnight. The excess of diazomethane was destroyed with acetic acid and the solution evaporated *in vacuo* to leave a red gum. Filtration of the gum through a short silica gel column yielded the *methyl ether* (4) (230 mg), which was crystallised from acetone, m.p. 139—140° (Found: C, 66.95; H, 5.7; N, 4.1. $C_{19}H_{19}NO_5$ requires C, 66.85; H, 5.6; N, 4.1%); $\nu_{max.}$ (KBr) 3 148, 3 116, 1 658, 1 620, 1 528, 1 200, 1 255, 1 152, 1 122, 1 090, 1 065, 950, 922, 840, 800, 765, and 740 cm^{-1}; *m/z* 341 (M^+, 100%), 340 (20), 327 (20), 326 (40), 298 (22), 296 (18), 280 (12), 272 (21), 268 (18), 43 (15), and 41 (15); for u.v. and ^{1}H n.m.r. data see Tables 1 and 2.

Isomerisation of tecleaverdoornine methyl ether (4).[11] Tecleaverdoornine methyl ether (4) (80 mg) was heated with a large excess of methyl iodide (4 g) in a sealed tube at 90—100 °C for 4 h. The excess of methyl iodide was distilled off and the residue dissolved in methanol, from which it crystallised (52 mg). Recrystallisation from methanol afforded *isotecleaverdoornine methyl ether* (10) as the hemihydrate, m.p. 169—170° (Found: C, 64.95; H, 5.95; N, 3.75. $C_{19}H_{19}NO_5$,0.5H_2O requires C, 65.1; H, 5.7; N, 4.0%); $\nu_{max.}$ (Nujol) 3 240, 1 605, 1 602, 1 550, 1 500, 1 350, 1 270, 1 240, 1 205, 1 140, 1 085, 1 020, 985, 940, 865, and 770 cm^{-1}; *m/z* 341 (M^+).

Attempted cyclisation of tecleaverdoornine with concentrated hydrochloric acid–acetic acid. Tecleaverdoornine (1) (200 mg) in glacial acetic acid (4 ml) and concentrated hydrochloric acid (37%; 1.5 ml) was heated on a steam-bath for 4 h. The mixture was then evaporated under reduced pressure. The greenish residue was treated with aqueous sodium carbonate (200 ml) and extracted with chloroform (4 × 20 ml). The extract was washed successively with sodium carbonate solution and water, dried (Na_2SO_4), and evaporated. The green gum obtained (t.l.c. showed a single spot) was dissolved in chloroform and filtered through a column of silica gel to give a solid (168 mg), which on crystallisation from acetone afforded the *chloro-compound* (5) as granular crystals, m.p. 183—184°, giving a deep green colouration with $FeCl_3$ (Found: Cl, 9.9. $C_{18}H_{18}ClNO_5$ requires Cl, 9.8%); $\nu_{max.}$ (Nujol) 3 400, 1 608, 1 510, 1 470, 1 355, 1 315, 1 265, 1 140, 1 105, 1 065, 1 040, 975, 930, and 840 cm^{-1}; *m/z* 363 (M^+, 20%), 327 (20), 312 (10), 273 (20), 272 (100), 259 (20), 285 (20), and 247 (23).

Attempted cyclisation of tecleaverdoornine with formic acid. Tecleaverdoornine (1) (100 mg) was treated with 98% formic acid (4 ml) and warmed on a water-bath for 30 min. The mixture was diluted with water and extracted with methylene chloride; the extract was washed with aqueous sodium carbonate, and then with water, dried (Na_2SO_4), and evaporated. T.l.c. revealed two spots. The residue was chromatographed on a silica gel column. Elution with hexane–diethyl ether (1 : 1) afforded tecleaverdoornine (1) in the first five fractions. Later fractions gave the *formyloxy-compound* (6) (65 mg) as colourless prisms from methanol, m.p. 187—188°; green colour with $FeCl_3$ (Found: C, 61.1; H, 5.35; N, 3.75. $C_{19}H_{19}NO_7$ requires C, 61.1; H, 5.15; N, 3.75%); $\nu_{max.}$ (Nujol) 3 200, 1 695, 1 600, 1 510, 1 375, 1 225, 1 160, 1 125, 1 040, 960, 930, 833, 815, and 780 cm^{-1}.

Hydrolysis of 8-hydroxy-5-(3-formyloxy-3-methylbutyl)-6,7-methylenedioxydictamnine (6). Compound (6) (20 mg) was treated with aqueous potassium hydroxide (10%; 5 ml) at room temperature for 1 h. The mixture was diluted with water and acidified with concentrated hydrochloric acid (2 ml). Neutralisation with ammonia solution gave a green precipitate, which was dissolved in methanol and refluxed with animal charcoal. Filtration, followed by concentration of the filtrate, gave granular crystals of *8-hydroxy-5-(3-hydroxy-3-methylbutyl)-6,7-methylenedioxydictamnine* (7) (12 mg), m.p. 217—218°, identical (m.p., mixed m.p. and i.r.) with tecleaverdine described below.

Tetrahydrotecleaverdoornine (11). Tecleaverdoornine (100 mg) in ethyl acetate (25 ml) was hydrogenated over palladium–charcoal (10%). Filtration (over Celite), evaporation, and recrystallisation of the residue from methanol–ethyl acetate yielded *tetrahydrotecleaverdoornine* (11) as colourless needles, m.p. 152—153° (Found: C, 65.0; H, 6.3; N, 4.65. $C_{18}H_{21}NO_5$ requires C, 65.25; H, 6.4; N, 4.25%); $\nu_{max.}$ (KBr) 3 310, 1 630, 1 530, 1 240, 1 180, 1 080, 1 000, 1 015, 963, 928, 800, 780, and 712 cm^{-1}; $\lambda_{max.}$ (MeOH) 233 ($\varepsilon_{max.}$ 31 600), 256 (45 700), 302 (6 300), and 327 nm (4 500); δ 0.95 (6 H, d, *J* 5.5 Hz), 1.40 (3 H, m), 3.00 (2 H, m), 3.60 (2 H, t, *J* 8 Hz), 4.10 (3 H, s), 4.65 (2 H, t, *J* 8 Hz), and 6.00 (2 H, s); *m/z* 331 (M^+, 60%), 275 (30), and 274 (100).

Hexahydrotecleaverdoornine (12). Tecleaverdoornine (100 mg) in glacial acetic acid was hydrogenated over Adams'

catalyst (50 mg). Filtration over Celite and evaporation yielded a white amorphous powder (90 mg) which was insoluble in all the usual solvents. It was washed several times with methanol and dried to give *hexahydrotecleaverdoornine* (12), m.p. >300 °C (Found: C, 64.55; H, 7.1; N, 4.0. $C_{18}H_{23}NO_5$ requires C, 64.85; H, 6.95; N, 4.2%); $\nu_{max.}$ (Nujol) 3 400, 1 638, 1 365, 1 310, 1 300, 1 270, 1 250, 1 230, 1 205, 1 129, 1 100, 1 055, 1 025, 925, 905, and 760 cm^{-1}; $\lambda_{max.}$ (MeOH) (qualitative since it was only sparingly soluble) 224, 245, 261, 305, 344, and 355 nm; remained unchanged on addition of acid: $\delta(C_5D_5N)$ 0.95 (6 H, d, *J* 7 Hz), 1.25—1.72 (6 H, m), 2.85—3.2 (4 H, m), 3.75 (3 H, s), and 6.10 (2 H, s); *m/z* 333 (M^+, 55%), 318 (25), 290 (80), 276 (100), 262 (35), 260 (25), and 246 (28).

Attempted isomerisation of tecleaverdoornine with methyl iodide.[11] Tecleaverdoornine (100 mg) and methyl iodide (4 ml) were heated in a sealed tube at 100 °C for 8 h. The tube was broken open and the excess of methyl iodide evaporated off. The residue crystallised from acetone to give colourless needles of the starting material (1) (m.p. 191 °C).

Attempted demethylation of tecleaverdoornine (1) *with ethanolic hydrochloric acid.* A solution of tecleaverdoornine (250 mg) in ethanol (5 ml) containing concentrated hydrochloric acid (10N; 5 ml) was refluxed on a water-bath for 20 h. The yellow solid which separated out on cooling was filtered off and treated with aqueous sodium hydroxide (10%). Acidification with acetic acid formed a precipitate (200 mg), which crystallised from acetone to give the chloro-compound (5), m.p. 183°.

Isolation of Tecleine (8).—The combined fractions 66—80 eluted by hexane–diethyl ether (3 : 2) and found by t.l.c. to be homogeneous were evaporated to dryness to leave a solid. Repeated washing with ethyl acetate furnished an amorphous solid (0.0015% with respect to dried bark) which was recrystallised from a large volume of hot ethanol to yield colourless needles of *tecleine* (8), m.p. 256—257° (lit.,[23] 256—257°); green colour with $FeCl_3$ and an emerald-green colour with a hot solution of gallic acid in concentrated sulphuric acid [5] (Found: C, 59.95; H, 3.35; N, 5.35. $C_{13}H_9NO_5$ requires C, 60.25; H, 3.45; N, 5.4%); $\nu_{max.}$ (KBr) 3 440, 3 164, 3 144, 1 610, 1 530, 1 485, 1 300, 1 240, 1 180, 1 160, 1 090, 1 035, 990, 965, 940, 873, 830, 780, 750, and 720 cm^{-1}; *m/z* 259 (M^+, 100%), 244 (60), 230 (20), 228 (28), 214 (91), 202 (40), 201 (36), 188 (36), 172 (80), 143 (95), 130 (60), and 78 (20); for ^1H n.m.r. and u.v. data see Tables.

Acetylation of tecleine (8). Tecleine (50 mg) was acetylated with pyridine (3 ml) and acetic anhydride (3 ml). The mixture was diluted with ice-cold water and extracted with chloroform (3 × 20 ml). The chloroform layer was dried (Na_2SO_4) and evaporated to leave a semi-solid, which crystallised from ethyl acetate to give colourless plates of *tecleine acetate* (9), m.p. 186—188° (Found: C, 59.55; H, 3.85; N, 4.3. $C_{15}H_{11}NO_6$ requires C, 59.8; H, 3.7; N, 65%); $\nu_{max.}$ (Nujol) 1 750, 1 605, 1 515, 1 475, 1 360, 1 320, 1 302, 1 230, 1 185, 1 085, 1 050, 1 035, 960, 925, 865, and 745 cm^{-1}; *m/z* 301 (M^+, 10%), 260 (18), 259 (100), 258 (16), 244 (20), 228 (18), and 214 (12); for u.v. and ^1H n.m.r. data see Tables 1 and 2.

Methylation of tecleine (8). Tecleine (40 mg) was methylated like tecleaverdoornine (1). Filtration of the methylated product through a column of silica gel afforded the *methyl ether* (2) (28 mg), m.p. 205—207° (from methanol), identical (mixed m.p., t.l.c. and i.r.) with flindersiamine (2) described in the next section.

Isolation of Flindersiamine (2) *and Kokusaginine* (14).—Fractions 103—120 eluted with hexane–diethyl ether (1 : 3) were combined and subjected to column chromatography over silica gel. Elution with hexane–diethyl ether (1 : 1) gave flindersiamine (2) in the early fractions as colourless needles (from methanol) (0.15%), m.p. 207—208° (lit.,[25] 206—207°), identical with an authentic sample. The free base was converted into its picrate with picric acid in propan-1-ol and crystallised from the same solvent as yellow needles, m.p. 200° (lit.,[25] 200—201°).

Isoflindersiamine. Flindersiamine (2) was heated in methyl iodide (4 ml) for 4 h at 100 °C. The product was crystallised from aqueous methanol; m.p. 209—210° (lit.,[25] 209—211°). Isoflindersiamine rapidly turned pink [25] on exposure to air.

Kokusaginine (14). Later fractions from the above column gave a white solid (0.01% with respect to dried bark), recrystallisation of which from methanol afforded kokusaginine as plates, m.p. 168—170° (lit.,[25] 168—169°), the spectra of which were identical with those reported for kokusaginine. Kokusaginine also readily formed a picrate on treatment with picric acid in ethanol; m.p. 216—217° (lit.,[25] 218.5—219.5°).

Tetrahydroflindersiamine (15). Flindersiamine (2) (1 g) in glacial acetic acid was hydrogenolysed over Adams' catalyst (100 mg). Filtration, evaporation, and chromatography of the residue over silica gel gave *tetrahydroflindersiamine* (15) (800 mg), which crystallised from methanol as columnar plates, m.p. 188—189° (Found: C, 60.25; H, 5.8; N, 4.6. $C_{14}H_{15}NO_5$ requires C, 60.65; H, 5.45; N, 5.05%); $\nu_{max.}$ (Nujol) 1 645, 1 618, 1 465, 940, and 923 cm^{-1}; $\lambda_{max.}$ (MeOH) 223 ($\varepsilon_{max.}$ 41 200), 241 (30 200), 292 (7 500), 341 (12 300), and 354 nm (10 000); the u.v. spectrum remained unchanged in acid solution; δ 1.18 (3 H, t, *J* 7.5 Hz), 2.67 (2 H, q, *J* 7.5 Hz), 3.83 (3 H, s), 4.10 (3 H, s), 6.00 (2 H, s), 6.80 (1 H, s), and 9.10 (1 H, br s, disappeared on deuteriation); *m/z* 277 (M^+, 90%), 276 (10), 262 (100), 248 (10), 247 (8), and 246 (12).

Isolation of Tecleaverdine (7).—Fractions 141—160, eluted by pure chloroform, on concentration gave a white solid (0.0015%) which was recrystallised from methanol to yield *tecleaverdine* (7) as colourless crystals, m.p. 218—219°, giving a deep green colour with $FeCl_3$ and an emerald-green colour with a hot solution of gallic acid in concentrated sulphuric acid (Found: C, 62.7; H, 5.55; N, 3.85%; M^+, 345.1208. $C_{18}H_{19}NO_6$ requires C, 62.6; H, 5.55; N, 4.05%; *M*, 345.1212); $\nu_{max.}$ (KBr) 3 420, 3 180, 3 160, 1 610, 1 530, 1 475, 1 268, 1 239, 1 188, 1 144, 1 118, 1 088, 1 060, 1 023, 1 000, 963, 930, 895, 870, 763, 750, and 715 cm^{-1}; *m/z* 345 (M^+, 75%), 327 (9), 312 (18), 273 (15), 272 (100), 259 (12), 257 (12), and 59 (15); for u.v. and ^1H n.m.r. data see Tables 1 and 2.

Direct Conversion of Tecleaverdoornine (1) *into Tecleaverdine* (7).[27]—Tecleaverdoornine (200 mg) was added to a solution of mercury(II) acetate (200 mg) in aqueous tetrahydrofuran (50%) and the mixture was stirred for 15 min at room temperature. Sodium hydroxide solution (3M; 5 ml) was added, followed by sodium borohydride solution (0.5N; 1 ml); a black deposit of mercury formed. Work-up in the usual way gave a residue which was dissolved in chloroform (10 ml), dried (Na_2SO_4), and chromatographed over a silica gel column. Elution with chloroform gave a solid (80 mg), m.p. 219°, identical with natural tecleaverdine and the hydrolysis product of the formyloxy-compound (6). Acidification of the aqueous layer left after separation of the

tetrahydrofuran layer with acetic acid followed by extraction with chloroform furnished more tecleaverdine (80 mg).

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REFERENCES

[1] F. R. Irvine, ' Woody Plants of Ghana,' Oxford University Press, Oxford, 1961, p. 488.
[2] Preliminary communication, J. I. Okogun and J. F. Ayafor, *J. Chem. Soc., Chem. Commun.*, 1977, 652.
[3] P. G. Waterman, *Biochem. Syst. Ecol.*, 1975, **3**, 149.
[4] V. U. Enyenihi, Ph.D. Thesis, University of Ibadan, 1975.
[5] A. Labat, *Bull. Soc. Chim. Fr.*, 1909, **5**, 451.
[6] A. W. Sangster and K. L. Stuart, *Chem. Rev.*, 1965, **65**, 69.
[7] L. H. Briggs, L. D. Colebrooks, H. M. Fales, and W. C. Wildman, *Anal. Chem.*, 1957, **29**, 904.
[8] A. V. Robertson, *Aust. J. Chem.*, 1963, **16**, 451.
[9] M. F. Grundon, D. M. Harrison, and C. G. Spyropoulos, *J. Chem. Soc., Perkin Trans. 1*, 1975, 302, and references cited therein.
[10] T. R. Govindachari, N. Wiswinathan, B. R. Pai, V. N. Ramachandran, and P. S. Subramanian, *Tetrahedron*, 1970, **26**, 2905.
[11] M. Terasaka, *J. Pharm. Soc. Jpn.*, 1933, **53**, 219.
[12] P. J. Scheuer in ' Chemistry of the Alkaloids,' ed. S. W. Pelletier, Van Nostrand Rheinhold, New York, 1970, p. 355.
[13] D. M. Clugson and D. B. Maclean, *Can. J. Chem.*, 1965, **43**, 256.
[14] E. Ritchie, W. C. Taylor, and J. C. Shannon, *Tetrahedron Lett.*, 1964, 1437.
[15] J. F. Collins, G. A. Gray, M. F. Grundon, D. M. Harrison, and C. G. Spyropoulos, *J. Chem. Soc., Perkin Trans. 1*, 1973, 94.
[16] E. A. Clarke and M. F. Grundon, *J. Chem. Soc.*, 1964, 4190.
[17] R. D. Brown and F. N. Lahey, *Aust. J. Sci. Res.*, 1950, **A3**, 615.
[18] F. N. Lahey, I. A. Lamberton, and J. R. Price, *Aust. J. Sci. Res.*, 1950, **A3**, 155.
[19] J. A. Lamberton and J. R. Price, *Aust. J. Chem.*, 1953, **6**, 66.
[20] S. R. Johns, J. A. Lamberton, and A. A. Sioumis, *Aust. J. Chem.*, 1968, **21**, 1897.
[21] M. O. Abe, Ph.D. Thesis, University of Ibadan, 1970, p. 53.
[22] R. M. Bowman and M. F. Grundon, *J. Chem. Soc. C*, 1966, 1504.
[23] R. R. Paris and A. Stambouli, *C. R. Acad. Sci.*, 1959, **248**, 3736.
[24] S. V. Binns, B. Halper, G. K. Hughes, and E. Ritchie, *Aust. J. Chem.*, 1957, **10**, 481.
[25] F. A. L. Anet, P. T. Gilham, P. Gow, G. K. Hughes, and E. Ritchie, *Aust. J. Sci. Res.*, 1952, **5A**, 412.
[26] M. F. Grundon in ' The Alkaloids,' Chem. Soc. Specialist Periodical Report, 1979, vol. 9, p. 78.
[27] P. G. Waterman, I. A. Meshal, J. B. Hall, and M. D. Swaine, *Biochem. Syst. Ecol.*, 1978, **6**, 239.