Afromontoside. A New Cytotoxic Principle from *Dracaena afromontana*

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A new cytotoxic principle, steroidal saponin afromontoside (**1**), has been isolated from a methanolic extract of the twigs of *Dracaena afromontana*. It was shown to be (25*R*)-furost-5-ene-3β,22α,26-triol 26-*O*-α-L-rhamnopyranoside 3-O-([*O*-α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside) by spectral and chemical methods. The aglycone of afromontoside, diosgenin, as well as dihydrodiosgenin and several structurally related compounds have been shown to be cytotoxic to cultured KB cells.

The roots and leaves of *Dracaena* species (Dracae-na: Greek female dragon, the juice when thickened is supposed to resemble dragon's blood [1]) have been used medicinally for a number of years, *e.g.*, for the treatment of rheumatism.[2] Since no work seemed to have been done previously on the active principles of *Dracaena afromontana*, we examined the twigs of this plant as a source of potential antineoplastic agents and isolated a new cytotoxic furostanol glycoside. The structural features in this molecule responsible for its cytotoxic activity have been investigated.

The methanol extract of the dried twigs of *Dracaena afromontana* showed significant inhibitory activity when tested *in vitro* against (KB) cells derived from human carcinoma of the nasopharynx.[3] As shown in the Scheme, the isolation of afromontoside (**1**) was guided by *in vitro* bioassay and was accomplished by three partitions, followed by crystallization of the active principle from acetone–methanol. Also isolated following extensive chromatographic purification was diosgenin, whose structure was confirmed by comparison with an authentic sample.[4]

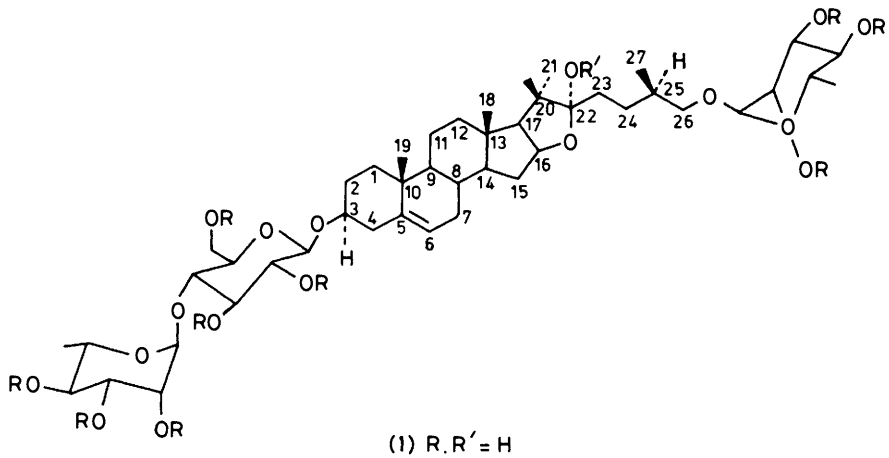
Structure of Afromontoside.—Afromontoside was obtained as colourless plates, m.p. >300 °C; $[\alpha]_D^{25}$ −69.3° (*c* 2.2, pyridine). The compound gave a positive test with reagents indicative of furostanol derivatives[5,6] and also gave positive Liebermann-Burchard and Molisch tests, consistent with the formulation of (**1**) as a steroidal glycoside.[7] The absence of the characteristic i.r. absorption in the region around 900 cm^{-1} (>915 cm^{-1}) suggested the absence of a spirostanol side chain.[8,9] When a solution of afromontoside (**1**) in absolute methanol was heated at reflux (15 h) it was converted into an OMe derivative (**2**), as judged by the disappearance of an OH resonance (δ 5.02) in the ^1H n.m.r. spectrum and the appearance of a signal (s, 3 H) at δ 3.15. When compound (**2**) was heated in aqueous acetone, it was converted back into (**1**); analogous interconversions have been noted between C-22 OMe and OH furostanol glycosides.[10,11]

Complete hydrolysis of afromontoside (0.1M-HCl, MeOH) afforded a glycosyl derivative of diosgenin (**3**), diosgenin (**4**), dehydrodiosgenin (**5**), D-glucose and L-rhamnose. The glycoside was identified as diosgenin 3β-glucoside (**3**) (trillin [12]) on the basis of its m.p. and spectral data, and by chemical transformation into the peracetate,[12] as well as further hydrolysis to diosgenin and glucose. The identity of the hydrolysis product (**4**) was established by direct comparison with an authentic sample of diosgenin; the spectral data were identical and no m.p. depression was observed upon admixture of the two samples. The successful conversion of (**1**) into (**3**) and (**4**), the last two compounds being of known configuration at C-3, C-20, and C-25, provided presumptive evidence for the absolute configuration of (**1**) at these asymmetric centres. The structure of the hydrolysis product (**5**) was determined by ^1H n.m.r. spectroscopy and by direct comparison of physical and spectral properties with those of an authentic sample obtained from diosgenin ($POCl_3$, pyridine). The observed hydrolysis pattern was consistent with that noted previously for other furostanol glycosides;[4,13–15] the attachment of D-glucose to C-3, rather than C-26, in afromontoside was suggested both by the formation of trillin (**3**) from afromontoside (**1**) in good yield and by the lack of hydrolysis of afromontoside by β-glucosidase.[14,15]

The negative chemical ionization mass spectrum of afromontoside had a molecular ion at *m/z* 885 ($M - H^-$) and a prominent peak at *m/z* 867 (loss of H_2O). Negative ions were also observed at *m/z* 721 and 576, reflecting the successive loss of two rhamnose moieties from (dehydrated) afromontoside. The positive CI mass spectrum of afromontoside peracetate had intense peaks at *m/z* 561 and 273, corresponding to peracetylated rhamnosylglucose and peracetylated rhamnose, respectively. The positive chemical ionization mass spectrum of the permethylate of afromontoside (**6**), prepared by the method of Hakomori,[16] had an ion at *m/z* 981 that was assigned as $(M + H - CH_3OH - CH_3)^+$. The base peak (*m/z* 189) and the ion at *m/z* 393 were attributed to loss of permethylated rhamnose and rhamnosylglucose moieties, respectively. Also present were the corresponding ions at *m/z* 836 and 633.

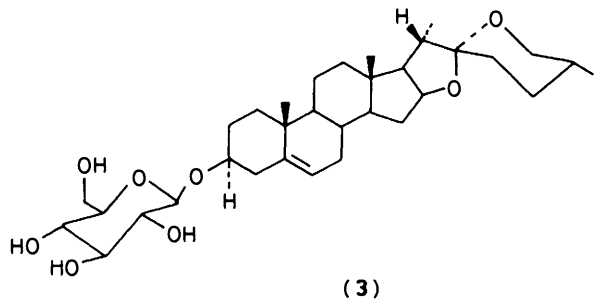
The loss of derivatized rhamnose and rhamnosylglucose moieties, but no trisaccharide, from the peracetylated and permethylated samples of afromontoside suggested that the three sugars present in (**1**) were not attached at a single site on the aglycone. This point was established definitively following complete hydrolysis of the permethylate (**6**), which provided diosgenin (**4**) and two methylated sugars. These sugars were identified as 2,3,6-tri-*O*-methyl-D-glucose [17,18] and 2,3,4-tri-*O*-methyl-L-rhamnose by comparison of chromatographic properties with those of authentic samples. Based on this data, the mass spectral fragmentation patterns, and the observed hydrolysis of (**1**) ⟶ (**3**), it was concluded that afromontoside contained a rhamnosylglucose group attached to the diosgenin nucleus at C-3 and an additional rhamnose moiety attached elsewhere to the aglycone. That this rhamnose was attached through C-26 of (**1**) was concluded from the observation that its hydrolytic removal resulted in formation of a spirostanol side chain ($\nu_{max.}$ 885 cm^{-1}; negative Ehrlich test).

The ^1H n.m.r. spectrum of the permethylate of afromontoside (**6**) showed three 1 H signals in the region δ 4.4—5.2, corresponding to the three anomeric protons of the sugars; these were at δ 5.22 (*J* < 2.5 Hz), 5.00 (*J* 3 Hz), and 4.40 (*J* 8 Hz). The last of these was assigned as the anomeric proton of a β-D-glucopyranosyl moiety, based on the appearance of analogous signals in the spectra of trillin (**3**) and its peracetate. The coupling constants observed for the remaining anomeric H's,[19]



(1) R,R′ = H
(2) R = H ; R′ = Me
(6) R,R′ = Me

0·1M-HCl | MeOH



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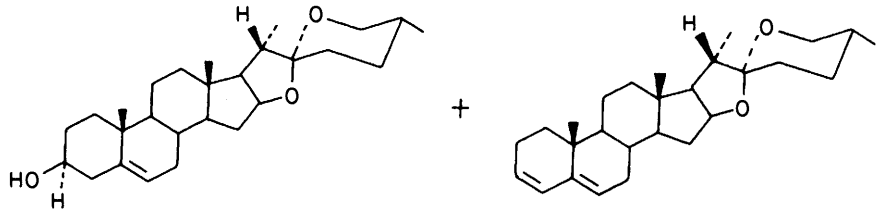


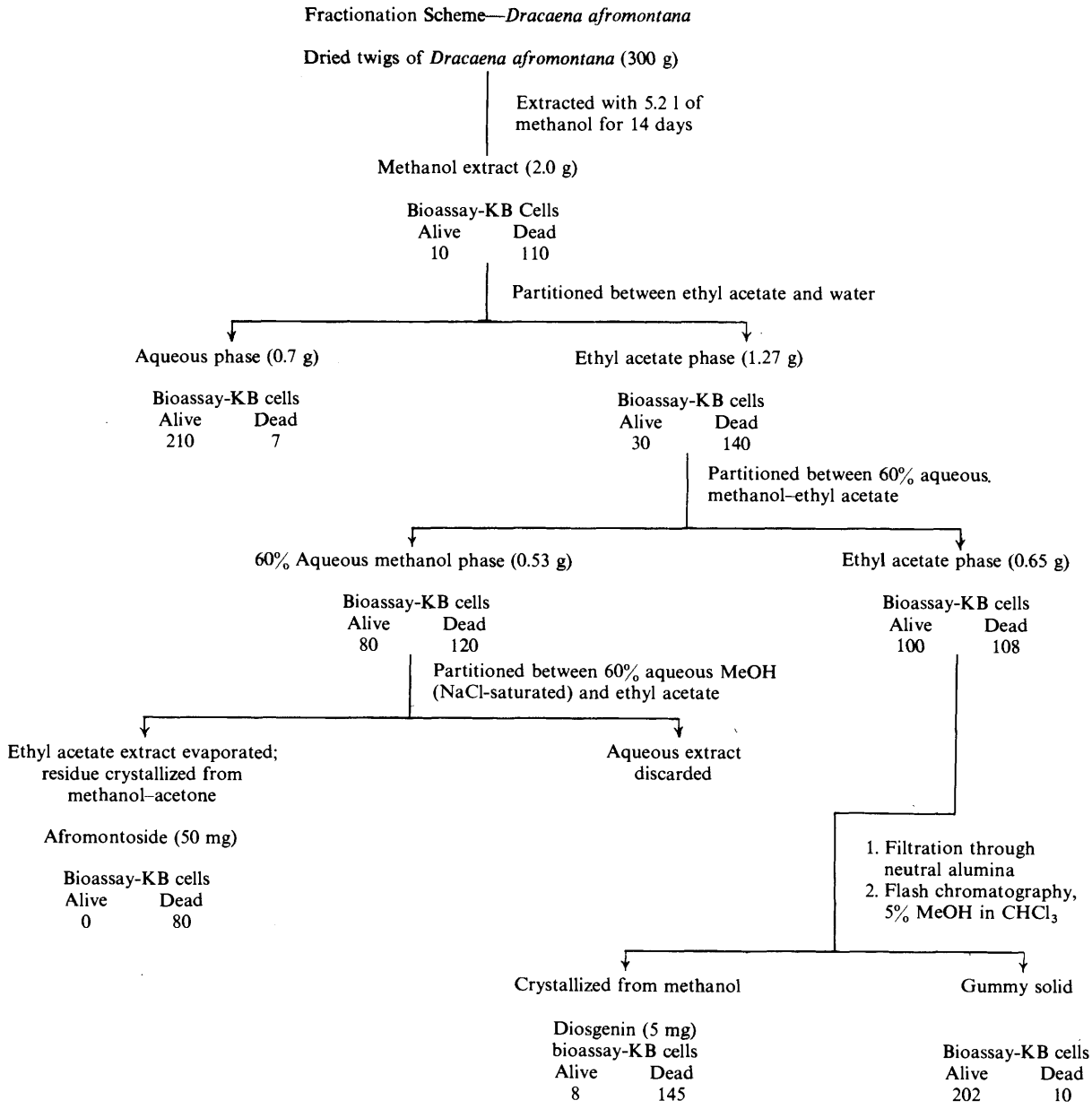
(4) + (5)

and the presence of common structural features in biosynthetically related compounds,[4,15,20,21] suggested that both L-rhamnose moieties in afromontoside had α-glycosidic bonds.

Further characterization of afromontoside as a putative furostanol derivative was attempted following treatment with $NaBH_4$.[22] After complete acid hydrolysis of the reaction mixture with methanolic H_2SO_4, two products were obtained. One of these was diosgenin; the other product (**7**) crystallized from acetone as colourless needles, m.p. 167—168 °C. ^1H N.m.r. and mass spectral data suggested that this compound was dihydrodiosgenin and it proved to be identical (mixed m.p., chromatographic properties) with an authentic sample of dihydrodiosgenin prepared from diosgenin.[23]

On the basis of the accumulated data, the structure of afromontoside was established as (25*R*)-furost-5-ene-3β,22α,26-triol 26-*O*-α-L-rhamnopyranoside 3-*O*-([*O*-α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside). Although there has been one recent example of a naturally occurring spirostanol steroidal saponin having L-rhamnose attached to ring F,[24] this is the first report of a furostanol glycoside having α-L-rhamnose at C-26. Also novel, as outlined below, is the finding of biological activity for this type of compound.

Biological Assays.—Afromontoside was isolated by following the cytotoxicity of fractionated material toward cultured KB cells.[3] The pure compound eliminated all viable cells, as judged by Trypan Blue exclusion,[25] when tested at a concentration of 100 μg/ml. It seemed of interest to attempt to identify the structural features in (**1**) that contributed to its cytotoxicity and several congeners were tested in the same fashion. Interestingly, while trillin (**3**) was essentially equipotent with afromontoside, both diosgenin (**4**) and dihydrodiosgenin (**7**) were significantly more active, producing toxicity (8—12 viable cells; 145—150 non-viable cells, relative to controls that contained 215—220 viable cells) at 10 μg/ml. This observation illustrated both that the sugar moieties were not required for expression of

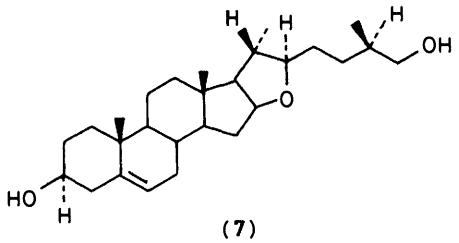


cytotoxicity and that there was little difference in activity observed between the furostanol and spirostanol derivatives. Dehydration of compounds (**4**) and (**7**) ($POCl_3$, pyridine) provided compounds (**5**) and (**8**), respectively. Assay of the dienes for cytotoxicity indicated that (**5**) was approximately as cytotoxic as (**4**), but that (**8**) was significantly more toxic (no viable cells, 13 nonviable cells) than (**7**) when tested at 10 μg/ml.

Epoxidation of compounds (**4**) and (**7**) with *m*-chloroperbenzoic acid provided the epoxides (**9**) and (**10**), respectively. Although the epoxide (**9**) was not observed to be active when tested at 10 μg/ml, both (**9**) and (**10**) killed all of the cells at a concentration of 100 μg/ml. Thus, while the epoxides were still cytotoxic to the cultured cells, epoxide (**9**) seemed to be less potent than structurally related spirostanols (**4**) and (**5**). In comparison, compound (**11**), which was obtained from diosgenin by treatment with Jones' reagent,[26] was found to exhibit significant cytotoxicity at 10 μg/ml (10 viable cells; 110 non-viable cells) and to kill all of the cultured cells when tested at 100 μg/ml. Thus it is clear that the pattern of substitution, and presumably functional group reactivity, substantially influences the cytotoxicity of this class of steroidal sapo(ge)nins.

Experimental

Melting points were measured on a Thomas Hoover Capillary melting point apparatus and are uncorrected. Column chromatography was carried out with Merck silica gel 60, particle size 0.0630—0.200 mm. T.l.c. monitoring of all reactions was performed with Merck silica gel 60 F254 pre-coated sheets (0.2 mm). Paper chromatography was performed on Whatman No. 1 paper, development with n-butanol–EtOH–H_2O (5:1:4). Chromatograms were visualized with aniline hydrogen phthalate spray, heated at 100 °C for 15 min. For sugars, t.l.c. was performed on cellulose plates (Eastman Chromagram), development with EtOAc–pyridine–H_2O (1:0.5:1, upper phase, developed twice). T.l.c. spots were visualized with $Ce(SO_4)_2$ (5%) solution, Ehrlich reagent (*p*-dimethylaminobenzaldehyde–HCl) and for sugars aniline hydrogen phthalate. U.v. spectra



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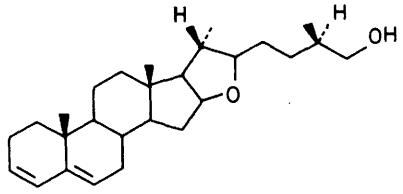

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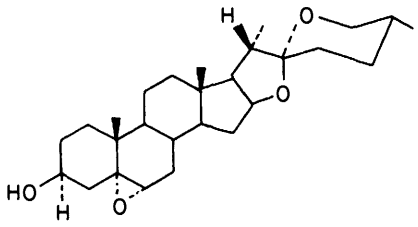

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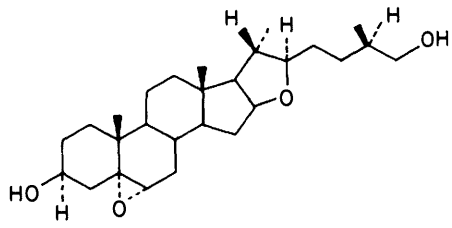

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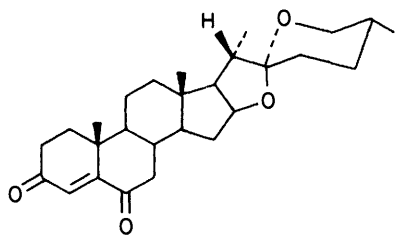

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were recorded on a Cary 15 Spectrophotometer; i.r. spectra with a Perkin-Elmer 257 Grating Spectrophotometer. ^{1}H N.m.r. spectra were recorded on a Nicolet NT 360 Spectrometer. All values are reported as p.p.m. downfield from $SiMe_4$. Electron impact mass spectral data were obtained on a Hitachi RMU-6E mass spectrometer; chemical ionization mass spectra were recorded on an AEI-MS 902 spectrometer. β-Glucosidase (emulsin) was obtained from Sigma Chemical Company.

Extraction and Fractionation of the Twigs of Dracaena afromontana.—The dried and ground twigs of *Dracaena afromontana* (300 g; collected from Kenya) were extracted successively with n-hexane, ether, methanol, and water at room temperature. Each extract was concentrated to dryness below 30 °C and tested for activity *in vitro* against Eagle's KB strain of human carcinoma of the nasopharynx. The methanol extract was found to be active; the other extracts were inactive and were not examined further. The green methanolic extract (2 g) was dissolved in water (80 ml). The aqueous phase was extracted with three 80-ml portions of ethyl acetate. The combined ethyl acetate extract was concentrated to dryness (< 30 °C bath-temp.) affording a pale green residue (1.27 g) that exhibited significant cytotoxic activity (see Fractionation scheme). This residue was partitioned between ethyl acetate (80 ml) and 60% aqueous methanol (80 ml). Each phase was found to have cytotoxic activity and each was examined separately. The residue obtained from the aqueous extract was again partitioned between 60% aqueous methanol (80 ml; fully saturated with NaCl) and ethyl acetate (80 ml); 10 ml of methanol was added to afford clear separation and to facilitate extraction of the active component into the organic phase. The ethyl acetate extract was concentrated and the residue was crystallized from methanol–acetone.

Afromontoside (**1**).—Crystallized from methanol–acetone (1:1) as plates, m.p. > 300 °C; $[\alpha]_D^{25}$ −69.3° (*c* 2.2, pyridine); $\lambda_{max.}$ 231 nm, $\nu_{max.}$ (KBr) 3 500—3 300, 1 645, 1 060, and 890—800 cm^{-1}; δ[$(CD_3)_2SO$] 0.79 (s, 3 H), 0.90 (d, 3 H, *J* 7 Hz), 1.05 (d, 3 H, *J* 7 Hz), 1.10 (s, 3 H), 1.14 (d, 3 H, *J* 6 Hz), 1.18 (d, 3 H, *J* 6 Hz), 2.4 (dd, 2 H, *J* 14.5, 5.5 Hz), 3.60 (m, 1 H), 4.0 (dd, 2 H, *J* 11, 4 Hz), 4.40 (d, 1 H, *J* 9 Hz), 4.60 (d, 1 H, *J* 4 Hz), 4.95 (d, 1 H, *J* 4 Hz), 5.02 (s, 1 H), and 5.32 (m, 1 H, *J* 5.5 Hz); *m/z* (CI − negative ion; CH_4/N_2O) 885 (M − H)$^-$, 867 (M − H_2O), 721, 576, and 412; R_F 0.45 [silica gel t.l.c.; development with $CHCl_3$–CH_3OH (65:35)] and R_F 0.33 (C-18 bonded reverse phase t.l.c.; development 9% H_2O in CH_3OH).

The peracetate of (**1**) was obtained by treatment of afromontoside (5 mg, 5.6 μmol) with pyridine (0.2 ml) and acetic anhydride (0.2 ml) at 25 °C for 12 h. The product (3.7 mg, 50%) was isolated as a white powder following extractive work-up and silica gel column chromatography (elution with 5% methanol in $CHCl_3$); (partial) ^{1}H n.m.r. ($CDCl_3$), δ 0.79 (s, 3 H), 0.98 (s, 3 H), 0.99 (d, 3 H, *J* 7 Hz), 1.02 (d, 3 H, *J* 6 Hz), 1.15 (d, 3 H, *J* 6 Hz), 1.18 (d, 3 H, *J* 6 Hz), 2.45 (m, 2 H), 4.40 (m, 1 H), 4.56 (d, 1 H, *J* 9 Hz), 4.82 (br s, 1 H), 5.25 (d, 1 H, *J* 4 Hz), 5.0—5.20 (m, 9 H), and 5.37 (m, 1 H); *m/z* (CI − positive ion) 975; *m/z* (CI − negative ion) 973, 561, 414, 397, and 273.

22-O-*Methyl Ether of Afromontoside* (**2**).—A solution of afromontoside (**1**) (10 mg, 11 μmol) in absolute methanol (10 ml) was heated at reflux for 15 h. The methanol was concentrated to a small volume and diluted with acetone (2 ml). The resulting precipitate was filtered off and dried, to afford (**2**) (8.0 mg, 78%) which was deposited as an amorphous solid from methanol, m.p. 250—252 °C (decomp); δ[$(CD_3)_2SO$] 3.15 (s, 3 H, OCH_3). Hydrolysis of (**2**) (5 mg) was accomplished by heating it at 100 °C in water (5 ml) for 16 h. The reaction mixture was concentrated to a small volume and treated with a few drops of acetone, which effected precipitation of afromontoside (3 mg), identical with an authentic sample as judged by ^{1}H n.m.r. spectroscopy and behaviour on silica gel t.l.c.

Hydrolysis of Afromontoside.—A sample of afromontoside (50 mg, 56 μmol) was heated at reflux with 2M-methanolic HCl (10 ml) for 5.5 h. The reaction mixture was cooled and the HCl was removed under a stream of nitrogen. The resulting residue was dissolved in methanol (10 ml) and maintained at room temperature overnight. Colourless needles of trillin (**3**) were filtered off (10 mg, 30%), m.p. 276—277 °C (lit.,[12] m.p. 275—280 °C); $[\alpha]_D^{25}$ −105° (*c* 1.1, dioxane); $\nu_{max.}$ (KBr) 3 500—3 200, 1 630, 975, 910 < 885 [(25R)-spirostanol skeleton], 850, 840,

and 820 cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ 0.7 (s, 3 H), 0.97 (d, 3 H, *J* 7 Hz), 0.99 (s, 3 H), 1.02 (d, 3 H, *J* 6 Hz), 3.45 (m, 1 H), 3.60 (dd, 1 H, *J* 14.5, 7 Hz), 3.75 (dd, 1 H, *J* 14.5, 3 Hz), 4.2 (d, 1 H, *J* 8 Hz), 4.45 (m, 1 H), and 5.32 (m, 1 H, *J* 5.5 Hz); *m/z* (CI − positive ion) 577 $(M + H)^+$, 561, 414, 396, 342, 282, 163, 139, and 115; R_F 0.5 [silica gel t.l.c.; development with $CHCl_3$–CH_3OH (65:35)].

The peracetate of compound (**3**) was obtained by treatment of (**3**) (10 mg, 17 μmol) with dry pyridine (0.2 ml) and acetic anhydride (0.2 ml) at 25 °C for 12 h. The product was purified by extractive work-up and crystallization from methanol as colourless plates, yield 8 mg (66%), m.p. 211—212 °C (lit.,[12] m.p. 202—203 °C); $\nu_{max.}$(KBr) 1 740, 1 635, 978, and 910 < 885 cm^{-1} δ($CDCl_3$) 0.79 (s, 3 H), 0.97 (d, 3 H, *J* 7 Hz), 0.99 (s, 3 H), 1.03 (d, 3 H, *J* 6 Hz), 2.0—2.1 (4 s, 12 H), 3.45 (dd, 2 H, *J* 11, 5.5 Hz), 3.65 (m, 2 H), 4.1 (dd, 1 H, *J* 14.5, 3 Hz), 4.25 (dd, 1 H, *J* 14.5, 5.5 Hz), 4.40 (m, 1 H), 4.6 (d, 1 H, *J* 8 Hz), 4.9 (t, 1 H, *J* 9 Hz), 5.07 (t, 1 H, *J* 9 Hz), 5.20 (t, 1 H, *J* 9.0 Hz), and 5.35 (d, 1 H, *J* 5.5 Hz); *m/z* (CI − positive ion) 745 $(M + H)^+$, 729, 684, 414, 396, and 331.

Compound (**3**) (5 mg, 8.7 μmol) was further hydrolysed by treatment with 5 ml of 0.1M-HCl in methanol at 90 °C for 1 h. Following extractive work-up, the products were identified as diosgenin (**4**) [colourless needles, m.p. 205—207 °C; lit.,[12] m.p. 206—208 °C; yield 2 mg (50%)] and D-glucose by direct comparison with authentic samples.

The mother liquor that deposited compound (**3**) from the hydrolystate of afromontoside (**1**) was partitioned between $CHCl_3$ and water. The $CHCl_3$ layer was washed with water and dried over anhydrous Na_2SO_4. The residue obtained following concentration of this layer was purified by silica gel column chromatography. Two crystalline compounds were isolated; these were identified as diosgenin (**4**) (5 mg, 20%) and dehydrodiosgenin (**5**) (2 mg, 9%) by direct comparison with authentic samples. The aqueous phase was heated at 80 °C for 1 h and tested for carbohydrates; only two sugars were detected, and these were identified as D-glucose and L-rhamnose by cellulose t.l.c. (R_F's 0.67 and 0.79, respectively, following development with ethyl acetate–pyridine–H_2O (1:0.5:1; upper phase) and paper chromatography [R_F's 0.10 and 0.29, development with n-butanol–acetic acid–water (5:1:4)] in comparison with authentic samples.

Diosgenin (**4**).—The residue obtained from the ethyl acetate layer following the ethyl acetate–60% aqueous methanol partition (see Fractionation scheme) was purified by filtration through alumina and subsequent flash chromatography[27] on silica gel. Crystallization from methanol afforded diosgenin (**4**) as colourless needles, m.p. 205—207 °C (lit.,[12] m.p. 206—208 °C); $[\alpha]_D^{25}$ −120° (*c* 1.18, $CHCl_3$); $\nu_{max.}$(KBr) 3 400—3 200, 1 650, 960, 950, 900 < 870, 840, and 810 cm^{-1}; mass spectrum, *m/z* 414 (M^+), 396, 342, 285, 139, and 115; R_F 0.7 [silica gel t.l.c.; development with $CHCl_3$–CH_3OH (5:1)]. The same compound was obtained from afromontoside (**1**) and trillin (**3**) by acid hydrolysis.

Dehydrodiosgenin (**5**).—Diosgenin (5 mg, 12 μmol) was dissolved in pyridine (0.2 ml) and treated with freshly distilled $POCl_3$ (2 drops). The reaction mixture was heated at 90 °C for 1 h and then cooled and poured over crushed ice. The product mixture was extracted with $CHCl_3$ and the $CHCl_3$ extract was washed successively with aqueous $NaHCO_3$ and water and then dried over Na_2SO_4. Concentration of the extract provided the crude product, which was precipitated from acetone as an amorphous powder (2 mg, 42%), m.p. 180—181 °C; δ($CDCl_3$) 4.40 (m, 1 H), 5.37 (m, 1 H), and 5.55 (m, 2 H); *m/z* 396 (M^+), 360, 324, 318, 303, 289, 267, 253, 213, and 139.

Enzymatic Hydrolysis of Afromontoside (**1**).—Afromontoside (10 mg) was dissolved in water (1.0 ml) and emulsin (β-glucosidase) (2 mg) was added. The mixture was incubated at room temperature for 3 days and then for an additional 3 days at 40 °C. Analysis of the product by t.l.c. showed no change in R_F value. The starting material (10 mg) was recovered unchanged after extractive work-up.

Methylation of Afromontoside and Identification of Methylated Sugars.—Sodium hydride (50% mineral oil dispersion; 125 mg, 2.6 mmol) was washed thoroughly with three 2-ml portions of n-hexane under N_2 and the resulting solid was heated at 58—60 °C with freshly distilled Me_2SO (2.5 ml) for 1 h. To this was added a solution of afromontoside (**1**) (10 mg, 11 μmol) in Me_2SO (1.25 ml) and the resulting mixture was stirred at room temperature for 1 h. Methyl iodide (2.0 ml) was then added in one portion and the reaction mixture maintained at room temperature overnight under nitrogen. The reaction mixture was poured into cold water and the product was extracted into $CHCl_3$ (3 × 10 ml). The $CHCl_3$ extract was washed with aqueous $Na_2S_2O_3$ and water, and dried over anhydrous Na_2SO_4. Methylated product (**6**) (8 mg, 70%) was obtained as a gummy solid after concentration of the $CHCl_3$ extract; the i.r. spectrum lacked OH absorption; δ($CDCl_3$) 3.4—3.6 (10 s, 30 H), 4.05 (m, 1 H), 4.40 (d, 1 H, *J* 8 Hz), 5.0 (br s, 1 H, *J* 3 Hz), 5.22 (br s, 1 H, *J* < 2.5 Hz), and 5.35 (m, 1 H); *m/z* (CI − positive ion) 981, 836, 807, 793, 647, 633, 619, 603, 585, 573, 535, 489, 471, 453, 425, 415, 397, 393, 283, and 189.

Hydrolysis of Permethylated Afromontoside.—Permethylate (**6**) was heated at reflux in HOAc–HCl–H_2O (1.5:3.5:5) (Killiani mixture) (10 ml) for 4 h. The reaction mixture was then concentrated to one-half volume, and the precipitated solid was filtered off and washed with water. The solid was crystallized from acetone, affording diosgenin (3 mg), m.p. 205—207 °C, which gave no depression upon admixture with authentic diosgenin. The filtrate was neutralized with an ion exchange resin and lyophilized. Analysis by silica gel t.l.c. [$CHCl_3$–CH_3OH (20:1)] indicated the presence of two components; these were separated by chromatography on a silica gel column (5 × 0.8 cm). Elution with $CHCl_3$ provided 2,3,4-tri-*O*-methyl-L-rhamnose (1 mg, 30%), identified by comparison with an authentic sample prepared from L-rhamnose. Further elution with $CHCl_3$–CH_3OH (20:1) gave 2,3,6-tri-*O*-methyl-D-glucose (0.5 mg, 29%), identified by paper chromatography [R_F 0.84, development with n-butanol–ethanol–H_2O (5:1:4)][18] and by direct comparison with an authentic sample prepared from D-maltose. The hydrolysis of the permethylate (**6**) with 5% methanolic H_2SO_4 (24 h, 25 °C) gave the same products.

Dihydrodiosgenin (**7**) *from Afromontoside* (**1**).—Afromontoside (10 mg, 11 μmol) was dissolved in distilled water (1.5 ml) and treated with $NaBH_4$ (6 mg, 15 μmol) at 20 °C for 24 h. To this was added 50% aqueous H_2SO_4 (0.5 ml) and the reaction mixture was heated at 100 °C for 7 h. The deposited precipitate was filtered off, washed with water, and the residue purified by silica gel column chromatography [elution with $CHCl_3$–CH_3OH (10:1)]. Compound (**7**) crystallized from acetone as colourless needles (2 mg, 43%), m.p. 167—168 °C; $\nu_{max.}$(KBr) 3 450—3 250, 1 620, 1 440, 1 370, 1 230, 1 090, 1 040, 1 000, 920, 870, 820, and 790 cm^{-1}; δ($CDCl_3$) 0.80 (s, 3 H), 0.92 (d, 3 H, *J* 7 Hz), 1.00 (d, 3 H, *J* 6 Hz), 1.02 (s, 3 H), 2.0 (dd, 2 H, *J* 12.5, 5.5 Hz), 2.3 (m, 2 H), 3.32 (m, 2 H), 3.5 (m, 2 H), 4.3 (m, 1 H), and 5.35 (d, 1 H, *J* 5 Hz); *m/z* (CI − positive ion) 417 $(M + H)^+$, 399 $(M - H_2O)^+$ 381, 329, 283, 271, 255, 243, 229, 149, 127, 109, 99, and 85.

Dihydrodiosgenin (**7**) *from Diosgenin* (**4**).—Anhydrous aluminium chloride (0.64 g, 7.5 mmol) in dry ether (10 ml) was

added to a cool (ice bath) mixture of lithium aluminium hydride (45 mg, 1.1 mmol) and dry ether (10 ml). A solution of diosgenin (50 mg, 0.12 mmol) in ether (10 ml) was then added with stirring over a period of 15 min. Stirring was continued for an additional 45 min at 0 °C and then for 2 h at reflux. The cooled reaction mixture was treated cautiously with water and dilute hydrochloric acid. The aqueous phase and suspended product were extracted with ether (4 × 10 ml) and the combined ether extracts were washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated to afford crystalline (**7**) (35 mg, 70%). Recrystallization from acetone provided (**7**) as colourless needles, m.p. 165—167 °C (lit.,[28] m.p. 167—168 °C).

Dehydrodihydrodiosgenin (**8**).—Compound (**8**) was prepared from dihydrodiosgenin (10 mg) in analogy with the conversion of (**4**) into (**5**). The product was obtained as a colourless oil following purification on a silica gel column (yield 6 mg, 63%); δ($CDCl_3$) 0.79 (s, 3 H), 0.95 (d, 3 H, *J* 7.2 Hz), 1.1—1.9 (m), 2.30 (m, 2 H), 3.35 (t, 2 H, *J* 11 Hz), 3.45 (m, 1 H), 4.4 (dd, 1 H, *J* 14.5, 7 Hz), 4.60 (m, 2 H), and 5.37 (d, 1 H, *J* 5.5 Hz); R_F 0.55 (silica gel, t.l.c. development with 5% methanol in $CHCl_3$).

Diosgenin 5α,6α-Epoxide (**9**).—Diosgenin (10 mg, 24 μmol) was dissolved in $CHCl_3$ (15 ml) and treated with *m*-chloroperbenzoic acid (6 mg, 35 μmol) in $CHCl_3$ (2 ml). The reaction mixture was maintained at room temperature for 24 h, then diluted with 20 ml of $CHCl_3$ and washed successively with 2% aqueous $NaHCO_3$, 1M-hydrochloric acid, and water. The organic phase was dried (Na_2SO_4) and concentrated under diminished pressure. Crystallization from acetone provided diosgenin epoxide (**9**) as colourless plates (7 mg, 68%), m.p. 185—186 °C; δ($CDCl_3$) 0.72 (s, 3 H), 0.78 (d, 3 H, *J* 7 Hz), 0.80 (s, 3 H), 0.95 (d, 3 H, *J* 7 Hz), 1.08 (s, 6 H), 2.9 (d, 1 H, *J* 5.5 Hz), 3.35 (t, 2 H, *J* 9 Hz), 3.45 (m, 2 H), 3.9 (m, 1 H), and 4.4 (m, 1 H); *m/z* 430 (M^+), 371, 361, 358, 316, 298, 287, 268, 251, and 139.

Dihydrodiosgenin 5α,6α-Epoxide (**10**).—The epoxide (**10**) was prepared from dihydrodiosgenin (**7**) (10 mg) in analogy with the conversion of (**4**) into (**9**). The product was obtained as colourless needles (acetone) (8 mg, 77%), m.p. 190—191 °C; *m/z* 432 (M^+), 414, 396, 373, 345, 327, 287, 269, and 251.

Acknowledgements

We thank Drs. W. S. Lynn and J. Campbell for some of the cytotoxicity results obtained with the crude extract of *Dracaena afromontana*.

References

1 L. H. Bailey, 'The Standard Cyclopedia of Horticulture,' Macmillan Company, New York, 1953, vol. 1, p. 1069.
2 J. M. Walt and M. G. Breyer-Brandwijk, 'The Medicinal and Poisonous Plants of Southern and Eastern Africa,' E. and S. Livingstane Ltd., London, 1962, p. 69.
3 V. I. Oyami and H. Eagle, *Proc. Soc. Exp. Biol. Med.*, 1956, **91**, 305.
4 G.-A. Hoyer, W. Sucrow, and D. Winkler, *Phytochemistry*, 1975, **14**, 539.
5 C. Sannie, S. Heitz, and H. Lapin, *C.R. Acad. Sci.*, 1951, **223**, 1670.
6 S. Kiyosawa, M. Hutoh, T. Kimori, T. Nohara, I. Hosokawa, and T. Kawaski, *Chem. Pharm. Bull.*, 1968, **16**, 1162.
7 L. F. Schneider, 'Qualitative Organic Microanalysis, Cognition and Recognition of Carbon Compounds,' Academic Press, New York, 1964, p. 172.
8 M. E. Wall, C. R. Eddy, M. L. McClennan, and M. F. Klumpp, *Anal. Chem.*, 1952, **24**, 1337.
9 C. R. Eddy, M. E. Wall, and M. K. Scott, *Anal. Chem.*, 1953, **25**, 266.
10 R. Tschesche, L. Seidel, S. C. Sharma, and G. Wulff, *Chem. Ber.*, 1972, **105**, 3397.
11 T. Kawasaki, T. Komari, K. T. Miyahara, T. Nohara, I. Hosokawa, and K. Mihashi, *Chem. Pharm. Bull.*, 1974, **22**, 2164.
12 R. E. Marker and J. Kreuger, *J. Am. Chem. Soc.*, 1940, **62**, 2548.
13 R. Tschesche and G. Wulff, *Fortschr. Chem. Org. Naturst.*, 1973, **30**, 461.
14 P. K. Kintya and G. V. Lazur'evskii, 'Steroid Glycosides of the Spirostan Series,' Kishinev, 1979, p. 62.
15 T. Nohara, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.*, 1975, **23**, 872.
16 S. Hakomori, *J. Biochem.* (*Tokyo*), 1964, **55**, 205.
17 P. K. Minocha, and K. P. Tiwari, *Phytochemistry*, 1981, **20**, 135.
18 R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, 1966, **44**, 249.
19 A. De Bruyn, M. Anteunis, R. De Gussem, and G. G. S. Dutton, *Carbohydr. Res.*, 1976, **47**, 158.
20 R. Hardman, J. Kosugi, and P. T. Parfitt, *Phytochemistry*, 1980, **19**, 698.
21 S. B. Mahato, N. P. Sahu, A. N. Ganguly, K. Miyahara, and T. Kawasaki, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2405.
22 R. Tschesche, G. Ludke, and G. Wulff, *Chem. Ber.*, 1969. **102**, 1253.
23 A. H. Albert, G. R. Pettit, and P. Brown, *J. Org. Chem.*, 1973, **38**, 2197.
24 K. Nakano, T. Nohara, T. Tomimatsu, and T. Kawasaki, *J. Chem. Soc., Chem. Commun.*, 1982, 789.
25 H. Sasamori, K. S. Reddy, M. P. Kirkup, J. Shabanowitz, D. G. Lynn, S. M. Hecht, K. A. Woode, R. F. Bryan, J. Campbell, W. S. Lynn, E. Egert, and G. M. Sheldrick, *J. Chem. Soc., Perkin Trans 1*, 1983, 1333.
26 R. E. Marker and E. Rohrmann, *J. Am. Chem. Soc.*, 1939, **61**, 1516.
27 W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.
28 G. R. Pettit and W. J. Bowyer, *J. Org. Chem.*, 1960, **25**, 84.

Received 3rd *October* 1983; *Paper* 3/1735