Structure Elucidation of Cordifolin A, a Novel Cucurbitacin from *Fevillea cordifolia*, using One and Two Dimensional N.m.r. Techniques

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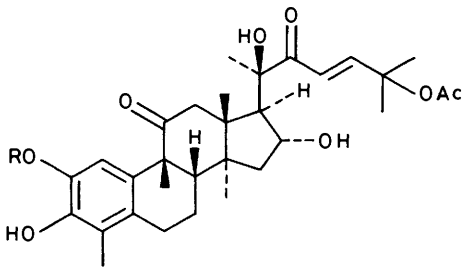
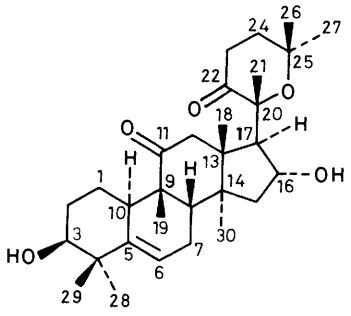
Cordifolin A (**3**) [3β,16α-dihydroxy-20,25-epoxy-19(10 ⟶ 9β)-*abeo*-10α-lanost-5-en-11,22-dione] was isolated from the methanol-soluble fraction of the 1% tartaric acid extract of dried seeds of *Fevillea cordifolia* L. (Cucurbitaceae) used in Jamaican folk medicine. Structure elucidation was based primarily on the use of multi-pulse n.m.r. techniques, especially combination of long range and direct $^1H,^1H$ INADEQUATE and ^1H, ^{13}C chemical shift correlation spectra. Biosynthesis of this novel triterpene apparently involves cyclization of the side chain to give a six-membered ether ring which is unprecedented among cucurbitacins.

The wide natural distribution and structural diversity of triterpenoids have evoked considerable interest in their chemistry and biological activity.[1,2] Many of the tetracyclic and pentacyclic triterpenes isolated from plants in the Cucurbitaceae family possess the biogenetically unusual 10α-cucurbit-5-ene [19(10 ⟶ 9β) *abeo*-10α-lanostane] skeleton, often in highly oxygenated form.[3,4] These compounds and their glycosides[5] have long been known to display a host of interesting biological activities which at least partly account for the extensive use of cucurbitaceaous plants in the folk medicine of most tropical and semi-tropical regions.[3,4] Recently there is renewed interest[6–13] in members of this class because of their cytotoxic and antitumour properties[8,14] anti-inflammatory[15] or antifertility activities,[16] immunostimulating effects,[17] sweet or bitter taste,[6,18] and potential as insect attractants[19] or plant growth regulators.[20]

Fevillea cordifolia L. (Cucurbitaceae) is a common dressing for wounds in Jamaican folk medicine, and has the reputation of being emetic, purgative, and highly toxic on ingestion.[21,22] Recent examination of the seeds of this plant led to the isolation of an unusual tetracyclic norcucurbitacin, fevicordin A (**1**), and its β-glucoside (**2**).[23] In the present report we describe the purification and structure elucidation of a novel pentacyclic triterpene, cordifolin A (**3**), from the seeds of *F. cordifolia*. Although ^1H and ^{13}C n.m.r. spectral assignments are available for the more common highly oxidized cucurbitacins,[3,7–9,18,24] application of multi-pulse n.m.r. methods[25,26] proved to be essential to obtain the structure of (**3**) which displays extensive resonance overlap.



(**1**) R = H
(**2**) R = β-D-glucosyl



(**3**)

Results and Discussion

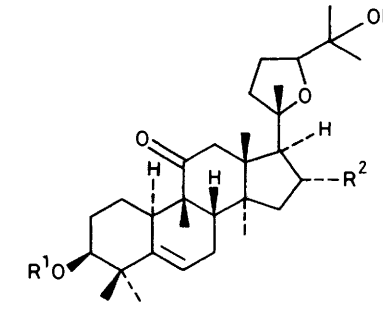
The methanol-soluble fraction of a 1% tartaric acid extract of the ground endosperm of dried *F. cordifolia* seeds was purified by high performance liquid chromatography (h.p.l.c.) and recrystallization (from methanol–water) to afford pure cordifolin A (**3**) in 0.03% yield. High resolution electron impact mass spectrometry suggested a molecular weight of 486.3352 and a molecular formula of $C_{30}H_{46}O_5$. Both fast atom bombardment (f.a.b-m.s.) and desorption chemical ionization (c.i.) mass spectra confirm the molecular weight, and exhibit an $[M + H]^+$ peak at *m/z* 487. Fourier transform i.r. spectroscopy suggests the presence of hydroxy, carbonyl, and olefinic functionalities with absorption bands at 3 390—3 560, 1 700, and 1 630 cm^{-1}, respectively. The ^1H n.m.r. spectrum in CD_3OD shows an olefinic proton, two methine protons, and eight methyl groups (Table 1). In conjunction with the attached proton test (APT),[27,28] the normal ^{13}C n.m.r. spectrum (Table 1) confirms the presence of 30 carbons (9 quaternary, 6 methine, 7 methylene, and 8 methyl). The ratio of carbons to hydrogen in the molecule indicates eight degrees of unsaturation. Since there are two olefinic signals (δ 120.34 and 141.42 p.p.m.), two carbonyl signals (δ 216.74 and 217.20 p.p.m.), and four resonances for carbons bearing singly-bonded oxygen in the ^{13}C n.m.r. spectrum, the combined data strongly suggest a pentacyclic triterpene in which the side chain has cyclized *via* an ether linkage. Although the cucurbitane skeleton appeared likely on the basis of previously-isolated natural products from this plant family, very few natural cucurbitacins contain a cyclic ether. Known examples include gratiogenin (**4**) and its derivatives (**5**) and (**6**),[29] cucurbitacin S (**7**),[30] and cucurbitacin T (**8**).[9]

The $^1H,^{13}C$ heteronuclear correlation (HETCOR) spectrum[25,26] of (**3**) identifies the specific resonances associated

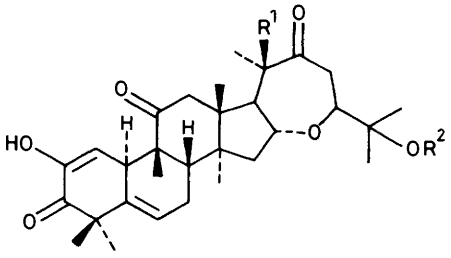
Table 1. ^{13}C and ^{1}H N.m.r. chemical shifts[a] of (3)

Carbon	Multiplicity	δ ^{13}C	δ ^{1}H
1	CH_2	21.67	1.32 (H_α), 1.56 (H_β)
2	CH_2	29.77	1.81 (H_α), 1.62 (H_β)
3	CH	76.90	3.43
4	C_q	42.31	
5	C_q	141.42	
6	CH	120.34	5.62 (d, *J* 5.6 Hz)
7	CH_2	24.77	1.92 (H_α), 2.38 (H_β)
8	CH	44.58	1.89
9	C_q	50.35	
10	CH	36.56	2.42
11	C_q	216.74	
12	CH_2	49.82	3.31 (H_α), 2.51 (H_β) (*J* 14.5 Hz)
13	C_q	49.21	
14	C_q	51.89	
15	CH_2	46.71	1.36 (H_α), 1.79 (H_β)
16	CH	71.50	4.41 (t, *J* 8.1 Hz)
17	CH	59.26	2.56 (d, *J* 7.3 Hz)
18	CH_3	20.41	0.89 (s)
19	CH_3	20.35	1.10 (s)
20	C_q	80.81	
21	CH_3	25.49	1.37 (s)
22	C_q	217.20	
23	CH_2	33.09	2.86 (H_α), 2.71 (H_β)
24	CH_2	38.11	1.71
25	C_q	70.81	
26	CH_3	29.39	1.18 (s)
27	CH_3	29.15	1.18 (s)
28	CH_3	28.21	1.04 (s)
29	CH_3	26.07	1.14 (s)
30	CH_3	19.75	1.31 (s)

[a] In p.p.m. from Me_4Si.



(4) R^1 = H R^2 = H
(5) R^1 = glucosyl R^2 = H
(6) R^1 = H R^2 = OH



(7) R^1 = H R^2 = H
(8) R^1 = OH R^2 = Me

with each protonated carbon (Figure 1). Proton-proton connectivities are available from the two dimensional proton-

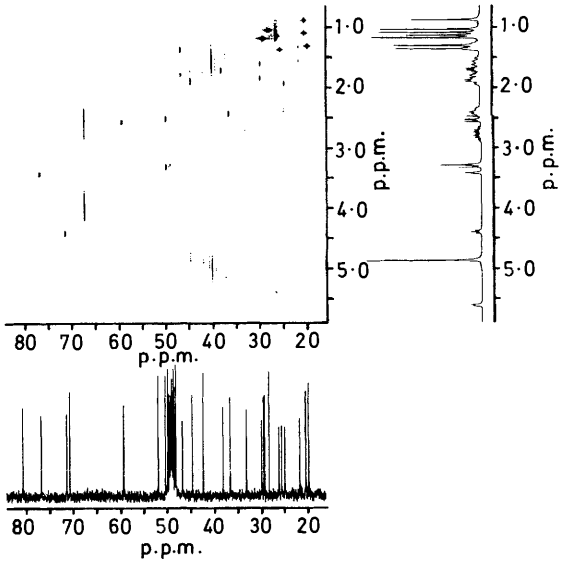


Figure 1. Two dimensional $^{1}H,^{13}C$ chemical shift correlation (HETCOR) spectrum of (3) in CD_3OD. The normal ^{1}H n.m.r. spectrum (300 MHz) is plotted along the vertical axis and the broad band ^{1}H-decoupled ^{13}C n.m.r. spectrum (75 MHz) is on the horizontal axis.

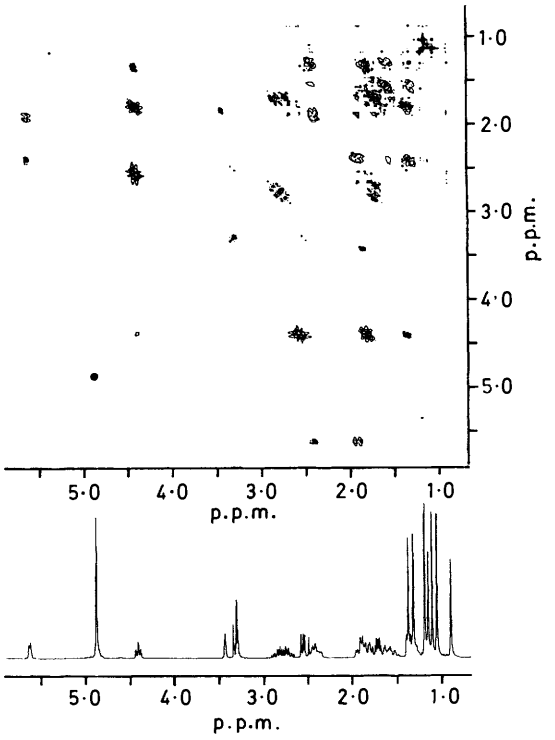


Figure 2. Two dimensional 300 MHz $^{1}H,^{1}H$ INADEQUATE spectrum of (3) in CD_3OD. A normal ^{1}H n.m.r. spectrum is plotted below.

proton INADEQUATE spectrum [31,32] wherein the off-diagonal cross peaks indicate the presence of mutually coupled hydrogens (Figure 2). By combining the information available from the two types of spectra, the methine carbons can be used as 'handles' for determining the connectivities of structural units within the molecule. Since the chemical shifts of the three methine carbons are significantly different (δ 36.56, 44.58, and 59.26 p.p.m.), these partial structures can be unambiguously assigned within the parent cucurbitane skeleton. Comparison with carbon resonance positions of known compounds [3] gives good agreement. Thus the HETCOR spectrum shows that the

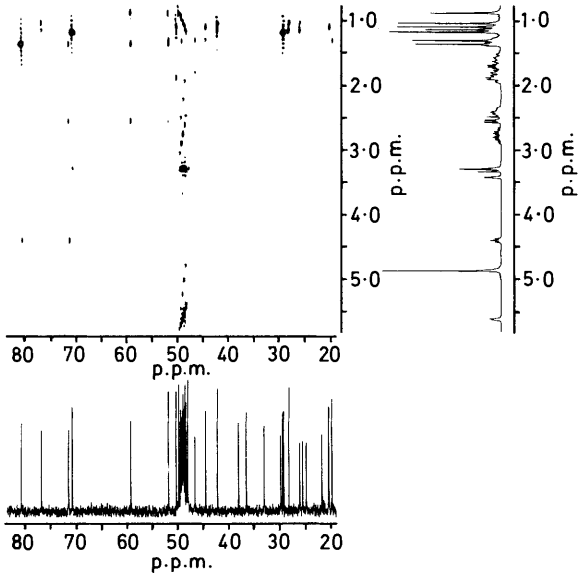


Figure 3. The ^{1}H-^{13}C long range correlation (COLOC) spectrum of (**3**). A broad band ^{1}H-decoupled ^{13}C n.m.r. spectrum (75 MHz) is plotted on the horizontal axis and a normal ^{1}H n.m.r. spectrum (300 MHz) is on the vertical axis.

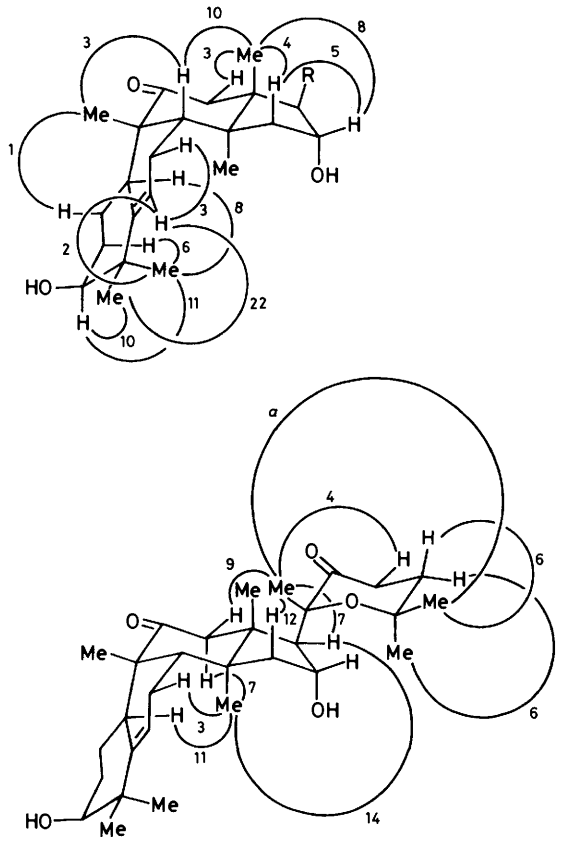


Figure 4. Interactions observed in difference n.O.e. experiments on (**3**) (two structures are shown for clarity). The numbers on the outside of the circles are percent enhancement upon irradiation of methyl groups (except for 6-H ⟶ 7-H and 16-H ⟶ 15-H). The exact value (*a*) of (21-H ⟶ 26-H) could not be determined in one dimensional experiments because of resonance overlap, but interaction was clearly seen in two dimensional n.O.e. spectra.

signal at δ 59.26 p.p.m. (C-17) bears the hydrogen which resonates at δ 2.56 p.p.m. From the ^{1}H,^{1}H INADEQUATE experiment it can be seen that this proton couples to another at δ 4.41 p.p.m., which from the HETCOR spectrum is attached to a carbon (C-16) at δ 71.50 p.p.m. bearing a hydroxy group. The proton at δ 4.41 p.p.m. also couples to the hydrogens at δ 1.36 and 1.79 p.p.m., which HETCOR reveals to be the methylenic pair at C-15 (δ 46.71 p.p.m.).[3,33] Similar connectivities and assignments can be established for: the ring B fragment consisting of C-8, C-7, and C-6; for a portion of ring A (C-10, C-1, C-2, and C-3); and for a section of the side chain (C-23 and C-24).

Table 2. Deuterium-induced isotope shifts of ^{13}C signals

Carbon	Δδ (p.p.m.)
25	0.105
16	0.103
3	0.113
20	0.090

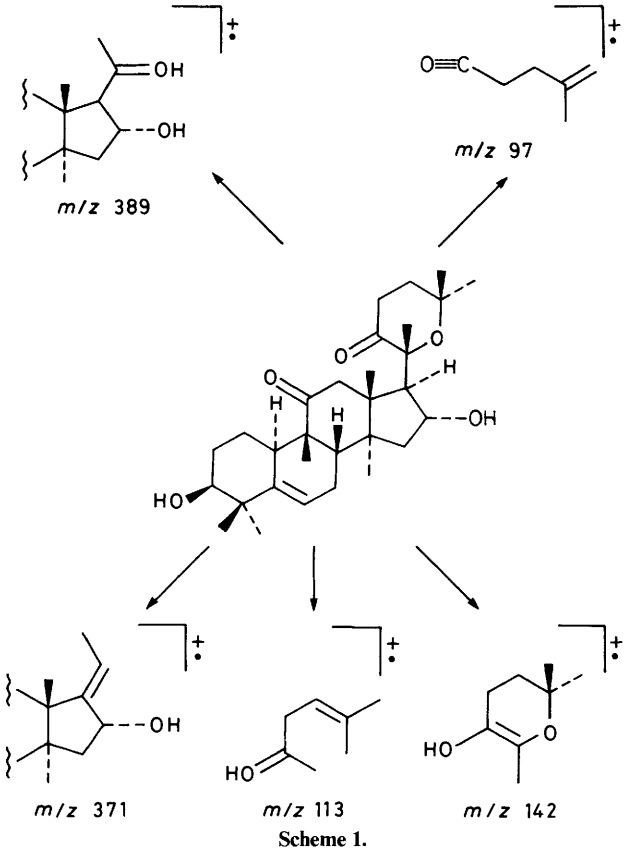
Correct union of these structural fragments as well as assignment of the intervening quaternary carbons and methyl resonances are made possible by the long range ^{1}H,^{13}C heteronuclear correlation (COLOC) spectrum[34] (Figure 3) and by a long range ^{1}H,^{1}H INADEQUATE experiment optimized for a proton-proton coupling constant of 1.56 Hz. For example, the COLOC spectrum of (**3**) confirms the position of the double bond by showing three bond couplings between C-5 and the methyl hydrogens at C-28 and C-29. This spectrum also shows that the hydrogens of these methyls couple with the quaternary C-4 carbon at δ 42.31 p.p.m. Similar interactions between the methylene protons at C-24 and the C-26 and C-27 methyl carbons, as well as between the corresponding methyl hydrogens and C-25, distinguish the geminal dimethyl groups of ring E from those of ring A. The remaining methyl and quaternary carbon signals are also readily identified except for C-13 (δ 49.21) and C-14 (δ 51.89), whose relative assignments are based primarily on literature analogy[3] because the COLOC experiments are compatible with either two or three bond interactions.

Observation of nuclear Overhauser effects (n.O.e.) by one dimensional difference spectroscopy[35,36] and two dimensional NOESY experiments[25,37] confirms the assignments and allows determination of the relative stereochemistry of (**3**). The significant enhancements are summarized in Figure 4. The key interactions which define the geometry of the ring junctures and of the E ring are obtained by irradiation of the C-28, C-19, C-18, C-30, and C-21 methyl groups. The overall stereochemistry of cordifolin A (**3**) is analogous to that of many cucurbitacins, but the appearance of a 6-membered cyclic ether in the side chain is unique.

An interesting phenomenon occurs upon partial exchange of (**3**) with a 1:1 mixture of deuteriated and undeuteriated methanol. The use of partial deuterium substitution of the hydroxy groups to cause induced isotope shifts in the ^{13}C n.m.r. spectrum of attached carbons is well established,[38] and such effects have also been seen for carbons bearing acceptor atoms for intramolecular hydrogen bonding.[39] Hence C-3 and C-16 were expected to be the two sites showing induced isotope shifts upon deuterium exchange. However, all four singly-bonded C–O resonances exhibit an induced isotope shift (Table 2). Molecular models show that the three dimensional arrangement of the C-16 hydroxyl and the ether oxygen in the E ring on the rigid tetracyclic framework do not allow direct intramolecular hydrogen bonding. Therefore the isotope shifts at C-20 and C-25 seem to indicate strong complexation of deuteriated solvent at the ether oxygen.[38]

The high resolution electron impact mass spectral fragmentation pattern confirms the structural assignment of cordifolin A

(3). The presence of the ether group and the 22-oxo functionality render the cyclised side chain particularly susceptible to cleavage. The intense peak at *m/z* 113.0968 ($C_7H_{13}O$, 100%) is characteristic for cucurbitacins bearing a saturated side chain with an oxygen at C-25, and has been suggested by Kupchan and co-workers[40] to arise by migration of the C-21 methyl group to C-22 and loss of the C-25 substituent. A related fragment at *m/z* 95.0862 (C_7H_{11}, 18%) is also observed. As shown in Scheme 1, the fragment ions at *m/z* 389.2693



Scheme 1.

($C_{24}H_{37}O_4$, 44%), *m/z* 371.2583 ($C_{24}H_{35}O_3$, 9%), and *m/z* 97.0655 (C_6H_9O, 16%) can also result from cleavage of bonds to C-20. The ketone at C-22 and the ether oxygen assist rupture of the bond from C-17 to C-20 with concomitant protonation to afford the characteristic side chain fragment[40] at *m/z* 142.0996 ($C_8H_{14}O_2$, 33%).

The relative stereochemistry of (3) agrees with the biogenetic considerations. Incorporation experiments reported with plant tissues and microsomes indicate that biosynthesis of cucurbitacins proceeds by cyclization of squalene oxide (9) to an enzyme-stabilized carbocation at C-9, which undergoes Wagner-Meerwein shifts to give 10α-cucurbita-5,24-dien-3β-ol (10) as the first cyclized compound (Scheme 2.[41,42] Other triterpenes which are common initial cyclization products (*e.g.* cycloartenol, parkeol, lanosterol) are not incorporated.[4,41,42] Oxidation of (10) at the sites usual for cucurbitacins (C-11, C-16, C-20, and C-22)[4] would yield a compound capable of Markovnikov addition of the C-20 hydroxy group to the side chain double bond to give (3). Further studies on the formation and biological activity of cordifolin A (3) and of other constituents of *F. cordifolia* are in progress.

Experimental

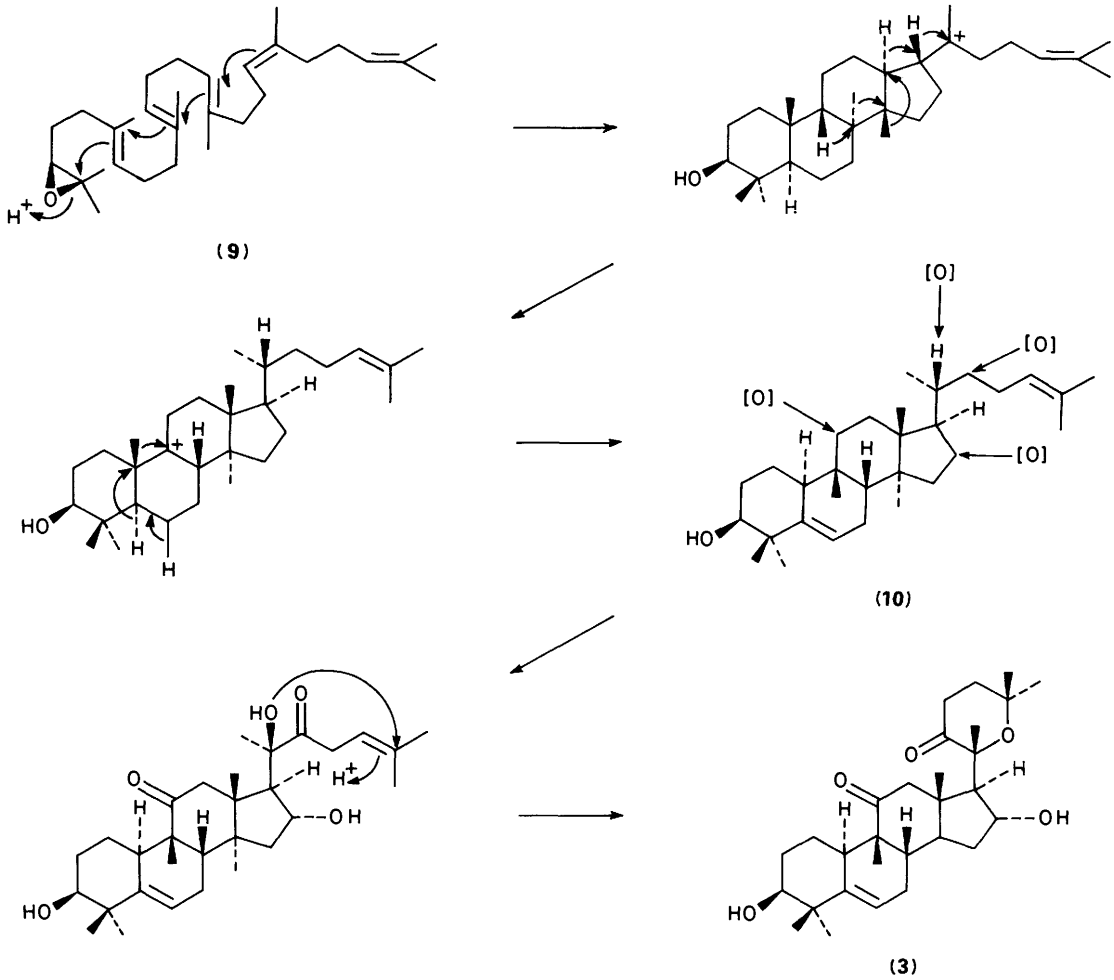
M.p.s were obtained on a Thomas-Hoover apparatus with open capillary tubes and are uncorrected. Methanol and acetonitrile were h.p.l.c. grade (Aldrich) and were used without further purification. Water was distilled, deionized (Millipore), and filtered (0.45 μm, Millipore) prior to use. H.p.l.c. separations employed a chromatograph (Perkin-Elmer) with the following components: a Series 3B solvent delivery system; LC-85 spectrometric detector and LC autocontrol; a computing integration system (C-R3A, Shimadzu); and a single-pen recorder (PM 8251, Philips). Sample injection was *via* a six-port manual injection valve (Rheodyne), equipped with either a 10 μl (analytical) or a 2.0 ml (preparative) sample loop. For analytical scale chromatography, a C_8 column (10 μm, 250 mm × 4.6 mm, Perkin-Elmer) was used. A column containing a similar stationary phase was used for the preparative scale separations (15 μm, 25.0 cm × 2.1 cm, Supleco). Column eluants were monitored at 254 nm using flow rates of 1.0 ml min^{-1} (analytical) and 10.0 ml min^{-1} (preparative). The mobile phase consisted of a linear stepwise solvent gradient of acetonitrile (A) and water [for analytical: 0 min (0% A), 15 min (0% A), 22 min (20% A), 40 min (40% A); for preparative: 0 min (0% A), 27 min (0% A), 40 min (20% A), 66 min (30% A), 75 min (90% A)]. Fraction collection was manual and where peak overlap occurred, the 'heart-cutting' method[43] was used. I.r. spectra were determined in KBr disc on a Nicolet 7199 FT-IR spectrometer. Mass spectra were obtained at an ionizing voltage of 70 eV on Kratos AEI instruments: MS-50 for high-resolution electron-impact ionization (e.i.); MS-12 for chemical ionization (c.i.); MS-9 for positive ion fast atom bombardment mass spectra (f.a.b.-m.s.).

Isolation of Cordifolin A (3).—The ground endosperm of dried seeds of *Fevillea cordifolia* (650 gm) was percolated overnight using 1% tartaric acid. The resulting solution was freeze-dried, redissolved in methanol, and filtered. The methanol-soluble extract (350 mg/ml) was purified using h.p.l.c. Fraction 30 (retention time 67.7 min) gave (3), which crystallized from the solution as white needles. It was further purified by recrystallization from methanol–water to afford pure (3) (160 mg, 0.03% yield), m.p. 135—136 °C; $[\alpha]_D^{22}$ +76.6 (*c* 1% in MeOH); $\nu_{max.}$ 3 390—3 560, 1 700 s, and 1 630 cm^{-1}; ^1H and ^{13}C n.m.r. are given in Table 1 (Found: M^+, 486.3352. $C_{30}H_{46}O_5$ requires *M*, 486.3345); both f.a.b.-m.s. (glycerol) and c.i.-m.s. (ammonia) show $(M + H)^+$ 487.

N.m.r. Methods.—N.m.r. spectra were recorded on a Bruker AM-300 instrument with solvent (referenced to tetramethylsilane) as internal standard for both ^1H [300.135 MHz; CD_3OD: δ 3.30 p.p.m.; $(CD_3)_2SO$: δ 2.49 p.p.m.) and ^{13}C (75.469 MHz; CD_3OD: δ 49.0 p.p.m.; $(CD_3)_2SO$: δ 39.5 p.p.m.) spectra. The attached proton test (APT) of (3) was done according to standard literature procedures.[27,28] Deuterium-induced isotope shifts[38] in ^{13}C n.m.r. spectra were measured in $(CD_3)_2SO$ on a sample of (3) which had been equilibrated with CD_3OD–CH_3OH (1:1).

The normal two dimensional $^1H,^1H$ INADEQUATE spectrum[31,32] (256 × 1 K) of (3) was obtained by accumulating 100 scans per t_1 value over a 1 572 Hz sweep width centred at δ 3.27. The relaxation delay was 0.3 s. The value of $J_{H,H}$ selected was 7.14 Hz. The data were zero-filled to 512 words in F1, subjected to Fourier transformation using Gaussian data manipulation in both dimensions, and symmetrized for improved appearance. The analogous long range INADEQUATE spectrum (256 × 2 K) was obtained by accumulating 500 scans per t_1 value over a 1 603 Hz sweep width centred at δ 3.21. The relaxation delay was 0.3 s. The value of $J_{H,H}$ selected was 1.56 Hz. The data was zero-filled to 1 K in F1, subjected to Fourier transformation using Lorentzian data manipulation, and symmetrized for improved appearance.

The two dimensional ^1H, ^{13}C heteronuclear shift correlation (HETCOR) spectrum[25,26] (241 × 2 K) of (3) for directly



Scheme 2.

bonded protons and carbons was obtained by accumulating 144 scans per t_1 value over 5 155 Hz sweep width centred at δ 50.2 (F2). The ^1H sweep width was 1 582 Hz, centred at δ 3.28 (F1). The relaxation delay was 1 s, and the value of $J_{C,H}$ selected was 135 Hz. The data were zero-filled to 512 in F1 and subjected to Fourier transformation using Gaussian data manipulation in F2. The long range two dimensional ^1H, ^{13}C heteronuclear shift correlation spectrum of (**3**) employed the COLOC pulse sequence.[34] A 72 × 2 K data matrix was generated accumulating 1 024 scans per t_1 value over 9 804 Hz sweep width centred at δ 80.4 (F2). The ^1H sweep width was 1 520 Hz, centred at δ 3.30 (F1). The relaxation delay was 0.5 s, and the value of $J_{C,H}$ selected was 5 Hz. The data were zero-filled to 512 in F1 and subjected to Fourier transformation using Gaussian data manipulation in both dimensions.

One dimensional n.O.e. difference experiments employed standard procedures.[35,36] The two dimensional n.O.e. (NOESY) spectrum[37] (228 × 1 K) of (**3**) was measured with 100 scans per t_1 value over a 1 572 Hz sweep width centred at δ 3.23. The relaxation delay was 1 s. The data were zero-filled to 1 K in F1, subjected to Fourier transformation using Lorentzian data manipulation in both dimensions, and symmetrized to improve appearance.

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