Pergamon

0031-9422(95)00703-2

Phytochemistry, Vol. 41, No. 4, pp. 1085–1092, 1996

0031-9422/96 $15.00 + 0.00

5,6-DIHYDRO-α-PYRONES FROM *SYNCOLOSTEMON PARVIFLORUS*

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(*Received* 19 *June* 1995)

Key Word Index—*Syncolostemon parviflorus*; Lamiaceae; 5,6-dihydro-α-pyrones; synparvolide A; synparvolide B; synparvolide C.

Abstract—The chemical structure and absolute stereochemistry of three new α-pyrones, synparvolides A-C, isolated from *Syncolostemon parviflorus*, have been established respectively as 6*R*-[5*S*,6*S*-(diacetyloxy)-1*R*,2*S*-(epoxy)-3*S*-(hydroxy)-heptyl]-5,6-dihydro-2*H*-pyran-2-one, 6R-[1*Z*,5*S*,6*S*-(diacetyloxy)-3*S*-(hydroxy)-1-heptenyl]-5,6-dihydro-2*H*-pyran-2-one and 6*R*-[6*S*-(acetoxy)-1*R*,3*S*-(dihydroxy)-2*R*,5*R*-(epoxy)-heptyl]-5,6-dihydro-2*H*-pyran-2-one, based on spectral, chiroptical, and chemical evidence.

INTRODUCTION

6-Substituted 5,6-dihydro-α-pyrones are an interesting group of biologically active natural products widely dispersed in the plant kingdom [1]. For several years we have been investigating two Southern African genera of the plant family Lamiaceae, *Syncolostemon* and *Tetradenia*, which have so far proved to be rich sources of these compounds [2–5]. One of the nine species included in the genus *Syncolostemon* [6], *S. parviflorus*, is a medicinal plant widely used by the Zulu people of Natal as an emetic to treat loss of appetite in both adults and children [7].

RESULTS AND DISCUSSION

Column chromatography of an acetone extract of the dried leaves of *S. parviflorus* gave oleanolic acid as the major constituent [2, 3]. Repeated flash chromatography of a polar column chromatography fraction yielded a yellow oil that gave a single spot on TLC. Examination of this oil by ^{1}H NMR spectroscopy revealed the characteristic triplet of doublets at δ6.9, assigned to the β proton of an α,β-unsaturated δ-lactone, and the other standard proton resonances indicative of this ring system [1]. However, it was also evident from the ^{1}H NMR spectrum that the oil was a mixture of α-pyrone compounds. Acetylation of a portion of this oil yielded an unstable crystalline residue which on GC analysis shortly after preparation showed the presence of two compounds, in an approximate 7:1 ratio, while GC analysis a month later revealed that the major compound had partially degraded on standing to yield a third compound. Several attempts to separate both the unacetylated and acetylated mixtures by flash chromatography on silica gel were unsuccessful. Eventually, however, the major compound, synparvolide A (**1**), $[\alpha]_D$ − 31°, and the minor compound, synparvolide B (**2**), $[\alpha]_D$ − 11°, were separated as oils by semi-preparative HPLC of the original unacetylated oil. A later collection contained chiefly synparvolide C (**3**), mp 115–115.5°, $[\alpha]_D$ + 17°, as well as smaller amounts of **1** and **2**.

The molecular formula of **2** was established as $C_{16}H_{22}O_7$ by HREI mass spectrometry. Unfortunately, **1** did not give a molecular ion in its EI mass spectrum but its low resolution CI (NH_3) mass spectrum contained a prominent peak at *m/z* 360 $[M + 1 + NH_3]^+$ in agreement with the molecular formula $C_{16}H_{22}O_8$.

The UV (λ_{max} *ca.* 205 nm, log ε 3.8) and IR spectra (ν_{max} 1710 cm^{-1}) of compounds **1** and **2** were consistent with those expected for an αβ-unsaturated-δ-lactone [1]. Further absorption bands at ν_{max} 3440 and *ca.* 1740 cm^{-1} were attributed to hydroxyl and acetate carbonyl stretching frequencies, respectively. The presence of these two functional groups was confirmed by the ^{1}H NMR spectra of **1** and **2** in which signals attributed to a single hydroxyl proton (δ *ca.* 3), two acetate methyls (δ *ca.* 2), a secondary methyl (δ1.2) and methylene (δ *ca.* 1.8) protons were observed. The only major difference between the ^{1}H NMR spectra of **1** and **2** was the presence of two oxymethine proton resonances at δ3.0 and 3.19 in **1** and two vinylic proton signals at δ5.55 and 5.61 in **2**. The ^{13}C NMR spectra of both **1** and **2** showed 16 well resolved resonances. The two signals at δ56.5 and 58.9 in

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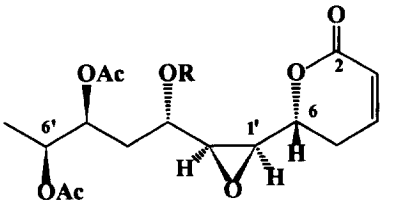

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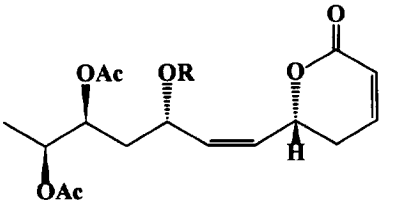
6 R = Ac



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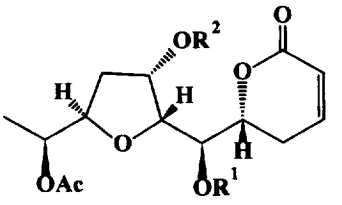
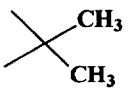
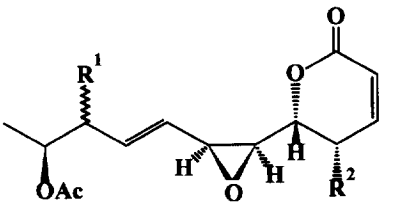
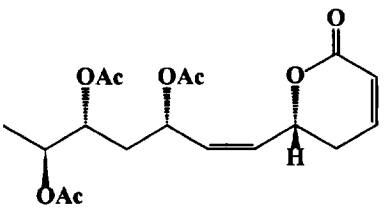


3 $R^1 = R^2 = H$
14 $R^1 = R^2 = Ac$
15 $R^1 + R^2 =$ 



4 $R^1 = \alpha$-OAc, $R^2 = H$
19 $R^1 = \beta$-OAc, $R^2 = \alpha$-OAc



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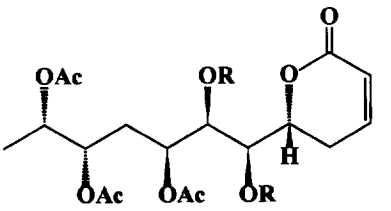
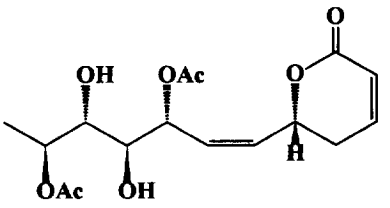


8 R = H
9 R = Ac



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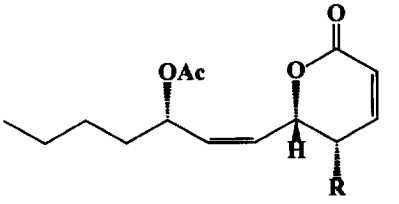
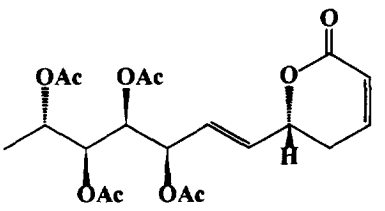


11 R = OAc
12 R = H

the ^{13}C NMR spectrum of **1** are in accordance with the values reported for the carbon atoms of the epoxide group in 5-deacetoxy-5′-*epi*-olguine (**4**) isolated from *Hyptis oblongifolia* (Lamiaceae) [8], thus establishing the presence of an oxirane ring in **1**. The position of the epoxide at C-1′, C-2′ in **1** and the double bond at the same position in **2** followed from COSY NMR experiments [9]. Further HMQC NMR experiments [10] enabled the unambiguous assignment of all the ^{13}C signals of **1** and **2**. The ^{13}C chemical shifts of both these compounds, consistent with those reported for two similar compounds, **4** [8] and hyptolide (**5**) isolated from *H. pectinata* [11], are presented in Table 1.

The COSY spectra of **1** and **2** suggested that both compounds possessed the same substitution pattern along the remainder of the acyclic side-chain but the exact positions of the two acetate groups were uncertain. Prominent correlations observed in the HMBC [12] spectra of both compounds between the C-5′ and C-6′ protons and the carbonyl atoms in the acetate moieties,



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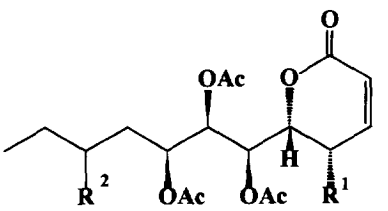
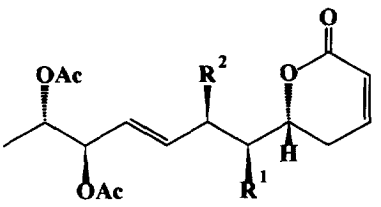


16 $R^1 = R^2 = H$
17 $R^1 = H$, $R^2 = OAc$
18 $R^1 = OAc$, $R^2 = H$



20 $R^1 = R^2 = OH$
21 $R^1 = OH$, $R^2 = OMe$
22 $R^1 = R^2 = OAc$

Table 1. ^{13}C NMR spectral data ($CDCl_3$) of compounds **1**, **2** (50 MHz), **3** (100 MHz), **4** (20 MHz) [8], **5** (125 MHz) [11] and **6–8**, **14**, **15** (100 MHz)

C	1	2	3	4	5	6	7	8	14	15
2	162.7	163.7	163.2	162.3	163.2	162.2	163.3	163.5	162.7	163.7
3	121.1	121.4	121.2	121.5	121.3	121.7	121.7	121.0	121.2	121.0
4	144.6	144.9	145.4	144.5	144.5	144.0	144.2	145.6	144.7	145.6
5	27.5	29.8	26.3	27.2	29.3	27.4	29.9	25.6	25.6	25.6
6	74.9	73.9	76.8	73.8	73.6	74.0	74.1	76.9	74.9	76.7
1′	58.9	135.8	71.5	57.9	131.0	57.6	130.4	72.1	70.8	69.9
2′	56.5	127.6	78.8	55.9	130.6	56.7	130.9	70.1	75.8	72.6
3′	64.6	64.6	73.7	127.6	66.3	66.4	65.7	70.3	73.3	70.3
4′	35.6	38.4	38.1	130.5	34.6	33.8	35.4	31.7	34.4	35.1
5′	71.0	72.0	81.4	74.3	70.7	70.7	70.7	71.3	78.3	79.7
6′	70.7	70.8	73.2	70.3	70.2	69.7	70.4	70.9	71.8	72.0
7′	16.0	16.2	16.7	15.3	14.5	16.1	16.1	16.2	16.0	16.1
Acetate Me	20.8	20.9	21.6	20.8	20.7	20.7	20.7	20.7	20.7	21.2
	21.0	21.0		20.9	20.8	20.8	20.9	21.0	21.0	
					20.8	21.0	21.0	21.1	21.1	
Acetate C=O	170.2	170.2	172.3	169.6	169.5	170.0	170.0	170.3	169.5	170.3
	170.8	171.6		169.9	170.4	170.2	170.2	170.5	170.3	
Acetonide C					170.8	170.3	170.3	171.8	170.4	98.2
Acetonide Me										19.2
										29.5

unequivocally placed these groups at C-5′ and C-6′ and hence the secondary alcohol at C-3′.

Mild acetylation of **1** and **2** gave the corresponding triacetates (**6**), $[\alpha]_D - 18°$, and (**7**), $[\alpha]_D + 27°$, as oils which also did not give molecular ions in their EI mass spectra. However, major peaks at *m/z* 402 $[M + 1 + NH_3]^+$ and 386 $[M + 1 + NH_3]^+$ in their respective CI mass spectra supported molecular formulas $C_{18}H_{24}O_9$ and $C_{18}H_{24}O_8$ for these two compounds.

Surprisingly, **6** was not very stable and the epoxide group slowly hydrolysed on standing in air, and rapidly on contact with silica gel, to give a mixture. The major product, isolated as a pale yellow oil by semi-preparative HPLC, was the diol triacetate (**8**), $[\alpha]_D + 1°$, which showed no duplication of ^{13}C NMR signals, suggesting that stereospecific acidic hydrolysis of **6** to **8** had occurred. A similar instability, and subsequent hydrolysis, of 3′-acetoxy-1′, 2′-epoxy-5,6-dihydro-α-pyrones has been reported [13]. The stability of the epoxide group in **1** relative to that in **6** may be due to stabilization through hydrogen bond formation between the vicinal hydroxyl proton on C-3′ and the epoxide oxygen in **1**. Acetylation of **8** gave the penta-acetate (**9**) as an oil, $[\alpha]_D + 5°$. A combination of COSY and HMQC

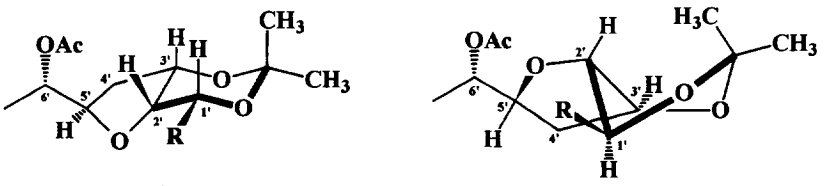


Fig. 1. Proposed conformations of the *syn*-1,3-diol acetonide (**A**) and the *anti*-1,3-diol acetonide (**B**) of **3**. R = 5,6-dihydro-α-pyrone ring.

experiments were used to assign the ^{13}C signals of compounds **6–8** which are shown in Table 1.

The *Z* configuration of the exocyclic double bond in **2** followed from the H-1′, H-2′ coupling constant (11 Hz) which is consistent with the literature values quoted for hyptolide **5** (10 Hz) [11], synrotolide (**10**) (11 Hz) from *S. rotundifolius* [2], pectinolide A (**11**) (11.1 Hz) from *H. pectinata* [13], and umuravarumbolide (**12**) (11 Hz) from *Tetradenia riparia* [5]. Unfortunately, the H-1′, H-2′ coupling constant for anamarine (**13**), the only 6-substituted 5,6-dihydro-α-pyrone isolated thus far from the Lamiaceae with $\Delta^{1'}$-*E* stereochemistry, has not been reported [14].

Synparvolide C (**3**), $C_{14}H_{20}O_7$, showed IR absorptions at ν_{max} 3440 and 1740 cm^{-1} due to hydroxyl and acetate groups. The ^{13}C spectrum of **3** revealed resonances consistent with an αβ-unsaturated-δ-lactone ring in addition to five oxymethine carbons (δ71–81), an acetate group (δ172 and 21), one methylene carbon (δ26) and one methyl carbon (δ16). ^1H NMR spectroscopy supported the presence of a single acetate group (δ2.1, *s*, 3H) and also revealed a dihydroxy component in the side chain with the collapse of two singlets (δ4.11 and 4.15) on the addition of D_2O. These data accounted for three of the five oxymethine functionalities and thus required a cyclic ether structure in the side chain of **3** to accommodate the remaining two oxymethine moieties.

Acetylation of **3** gave the expected triacetate (**14**) and a series of 2D NMR experiments enabled complete assignment of the ^{13}C and ^1H signals in both these compounds (see Table 1). The downfield chemical shifts of H-1′ and H-3′ (δ5.1 and δ5.4) in **14** suggested that hydroxyl groups situated at C-1′ and C-3′ had been acetylated. HMBC correlations between H-1′, H-3′ and H-6′ and acetate carbonyl carbon atoms confirmed the acetylation pattern as shown in **14**. A further prominent HMBC correlation between H-2′ and C-5′ unequivocally established the size and position of the five membered heterocyclic ring in the side-chain. The dispersal of the oxymethine signals in the ^1H NMR spectrum of **14** made this compound amenable to NOE difference experiments and this technique was used to explore the relative stereochemistry of the cyclic ether moiety. Thus irradiation of H-2′ gave significant enhancement of H-3′ and no increase in H-5′ which confirmed the *cis*-orientation of H-3′ relative to H-2′ and the *trans* configuration across the oxygen bridge. The 1′,3′-*syn* diol structure (**3**) is supported by the ^{13}C NMR spectrum of the crystalline isopropylidene derivative (**15**) [15] (see Table 1) which indicates that it possesses a chair conformation with the two alkyl substituents equatorial and the one methyl group axial (δ19) and the other equatorial (δ30). The two methyls in the isopropylidene derivative for the *anti*-1,3-diol would both appear at about δ25 in the twist boat conformation of the 6-membered ring [16] (see Fig. 1).

The absolute stereochemistry at C-6 in **1–3** was established by CD measurements. Thus the positive $n \rightarrow \pi^*$ Cotton effect observed in the spectra of **1** (λ_{max} = 255 nm, $\Delta\varepsilon$ = + 2.37), **2**(λ_{max} = 258 nm, $\Delta\varepsilon$ = + 2.97) and **3** (λ_{max} = 256 nm, $\Delta\varepsilon$ = + 3.4), together with the pseudo-equatorial orientation of the side chain at C-6 ($J_{6,1'}$ 8, 6.6 and 4.3 Hz respectively), indicates a (6*R*)-configuration for these compounds [1]. This stereochemistry is the same as that found in all previous 5,6-dihydro-α-pyrones isolated from the Lamiaceae (Table 2). Attention must be drawn to a serious error in our review [1]. The structures **82** and **83** on page 25 give rise to negative and positive Cotton effects respectively and not the other way round.

The determination of the (*R*)-configuration at C-6 in **1** by chiroptical analysis facilitated the tentative assignment of the stereochemistry at the adjoining pair of contiguous chiral centres from their ^1H NMR coupling constants. The H6, H1′ coupling constant (8 Hz) and the H1′, H2′ coupling constant (4 Hz) of **1** are identical to the values reported for **4** [8], suggesting the same (1′*R*,2′*S*)-stereochemistry in **1**. Compound **9** is derived from **1** and proved amenable to extensive ^1H NMR decoupling experiments the results of which further corroborates this stereochemical assignment. The coupling constants of the protons at C-6, C-1′, C-2′ and C-3′ of **9** ($J_{6,1'}$ = 6.9 Hz, $J_{1'2'}$ = 3.6 Hz, $J_{2'3'}$ = 6.1 Hz) are consistent with those of boronolide (**16**) ($J_{6,1'}$ = 5.7 Hz, $J_{1'2'}$ = 5.2 Hz, $J_{2'3'}$ = 5.5 Hz) in which the stereochemistry at the four contiguous chiral centres has been established by chemical degradation [3]. A similar comparison of relevant observed and calculated coupling constants has been used to assign the absolute configuration at C-1′–C-3′ in syndenolide (**17**) [4] and the tetraacetate (**18**) derived from acetylation of the major vicinal diol product from an attempted epoxidation of **11** [13].

The absolute stereochemistry of the chiral secondary hydroxy group at C-3′ in synparvolide A was also determined by the modified Mosher's method. Thus, **1** was converted to the (*S*)- and (*R*)-MTPA esters [17] and from the ^1H NMR spectra of these derivatives $\Delta\delta_H$-values ($\delta_{H(S)}$–$\delta_{H(R)}$) were calculated for all protons in each compound. From the MTPA determination rule [18] the

Table 2. The absolute stereochemistry of 6-heptyl 5,6-dihydro-α-pyrones isolated from the Lamiaceae

Compound	Chiral centres								
	5	6	1′	2′	3′	4′	5′	6′
4 [8]	—	(*R*)	(*R*)	(*S*)		*E*	(*R*)	(*S*)
5 [11]	—	(*R*)	*Z*		(*S*)	—	(*R*)	(*S*)
10 [2]	—	(*R*)	*Z*		(*R*)	(*R*)	(*S*)	(*S*)
11 [13]	(*S*)	(*S*)	*Z*		(*S*)	—	—	—
12 [5]	—	(*R*)	*Z*		(*S*)	—	—	—
13 [14]	—	(*R*)	*E*		(*R*)	(*S*)	(*S*)	(*S*)
16 [3]	—	(*R*)	(*R*)	(*R*)	(*S*)	—	—	—
17 [4]	—	(*R*)	(*R*)	(*R*)	(*S*)	—	?	—
19 [19]	(*S*)	(*R*)	(*R*)	(*S*)		*E*	(*S*)	(*S*)
20 [8]	—	(*R*)	(*S*)	(*R*)		*E*	(*R*)	(*S*)
21 [8]	—	(*R*)	(*R*)	(*R*)		*E*	(*R*)	(*S*)
22 [8]	—	(*R*)	(*R*)	(*R*)		*E*	(*R*)	(*S*)

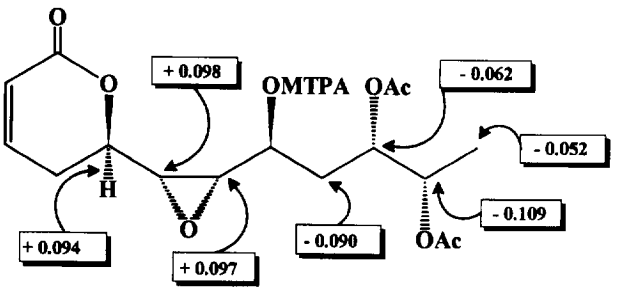


Fig. 2. Determination of absolute configuration at C-3′ in the MTPA ester of compound **1** (ΔS values in p.p.m).

positive and negative $\Delta\delta_H$-values observed for the signals of the protons in the left and right segments, (see Fig. 2), respectively, indicated a (3′*S*)-stereochemistry for **1** which agrees with that proposed from the proton coupling constants. The (3′*S*) -stereochemistry predominates in the 5,6-dihydro-α-pyrones isolated thus far from the Lamiaceae (see Table 2) with only **10** and **13** appearing to be the exceptions. However, closer examination of these two compounds reveals that their configuration at C-3′ is effectively the same as in compounds **5**, **11**, **12**, **16** and **17** and differs only because of a Cahn-Ingold-Prelog order priority reversal brought about by an oxygen containing functional group replacing a methylene group at C-4′ in **10** and **13**. A (3′*S*)-configuration is also probably present in synparvolide B but could not be confirmed due to lack of material.

There are only minor differences in the ^{1}H NMR spectra of **7** and **5** (see Table 3) but their optical rotations differ significantly, suggesting a diastereomeric relationship between them. Assuming both **7** and **5** have a (6*R*)- and (3′*S*)-configuration, stereochemical differences are limited to the remaining chiral centres at C-5′ and C-6′. Table 2 shows that all 6-substituted 5,6-dihydro-α-pyranones isolated previously from the Lamiaceae possess a (6′*S*)-stereochemistry. Accordingly **7** is also likely to contain (6*S*′)-stereochemistry and hence the stereochemical difference between **7** and **5** is confined to C-5′. Since hyptolide (**5**) has been proven [1] to be (5′*R*,6′*S*), **7**, and also **2**, must possess a (5′*S*,6′*S*)-stereochemistry. Synparvolide B (**2**), is the logical biosynthetic precursor of synparvolide A (**1**), with epoxidation occurring at the least hindered face of the exocyclic double bond [13], and therefore the proposed absolute configuration at C-5′ and C-6′ in **2** should also apply to **1**. X-ray crystallography would definitively establish the stereochemistry at C-5′ and C-6′ in **1** or **2** but all attempts to obtain suitable crystals of these compounds or their derivatives have thus far proved unsuccessful.

The configuration of the only remaining unknown chiral centre in synparvolide C, that at C-6′, was determined as follows. Saponification followed by acetonide formation of **3** gave a product possessing a ^{1}H NMR spectrum very similar to that of synparvolide C acetonide excepting that the acetate methyl signal at δ2.05 was missing, indicating that acetonide formation had as expected only occurred with the C-1′ and C-3′ hydroxyl groups. The chirality of the secondary hydroxyl group at C-6′ was then proved by Mosher's method. The reaction mixture could not be worked up as usual because of the presence of the acid-labile acetal group and after simple treatment with water the product was separated by chromatography. From the Mosher determination rule [18] the $\Delta\delta_H$ value ($\delta_{H(S)} - \delta_{H(R)} = -0.103$) for the C-7′ methyl group clearly showed that C-6′ has a (*S*) configuration.

Table 3. ^{1}H NMR spectral data of compounds **5** [11] and **7**

H	5*	7†
3	6.04 *dt* (10, 1, 1)	6.04 (9, 8, 1, 1)
4	6.90 *dt* (10, 6, 3)	6.87 *dt* (9.8, 6, 2.5)
5a‡	2.40 *m*	2.31 *m*
5e‡	2.45 *m*	2.49 *m*
6	5.30 *dt* (12, 7, 4.5)	5.37 *dt* (11.6, 7.8, 4.2)
1′	5.78 *dd* (10, 7)	5.70 *dd* (10.8, 7.8)
2′	5.54 *m*	5.52 *m*
3′	5.54 *m*	5.48 *m*
4′a	1.84 *m*	1.79 *m*
4′b	2.02 *m*	1.92 *m*
5′	4.94 *m*	4.96 *m*
6′	5.00 *dq* (6.5, 2.9)	5.07 *m* (6.5, 4.5)
7′	1.21 *d* (6.5)	1.18 *d* (6.5)
Ac	2.03 2.05 2.08	2.03 2.04 2.08

Values in $CDCl_3$; coupling constants (Hz) in parentheses.
*Spectrum obtained at 500 MHz.
†Spectrum obtained at 400 MHz.
‡Assignments interchangeable.

Monodesacetyl-synparvolide A (**23**) is probably the precursor of synparvolide C (**3**) and a plausible biosynthetic pathway is shown in Fig. 3. The stereochemistry of **3** is matched by that in **1** excepting for the incipient electrophilic centre at C_2' in **1** where inversion occurs during nucleophilic attack by the C-5′ oxygen atom. Synparvolide C is the first example of a 6-substituted 5,6-dihydro-α-pyrone containing a cyclic ether moiety in the C-6 side chain.

EXPERIMENTAL

The NMR spectra were run on Bruker 200 and 400 Mz instruments and the IR spectra run neat on a NaCl disc. A Whatman Magnum 9-Partisil 10 column was used for semi-prep. HPLC and a Durabond fused silica capillary column (coated with DB 17) with an FID detector were used for the GC analyses. The first collection of *Syncolostemon parviflorus* was made at Botha's Hill between Durban and Pietermaritzburg in April 1988 (Natal University voucher no. Edwards and Browning 105). The second collection during July 1994 came from the Vernon Crookes Nature Reserve (Natal University voucher no. Edwards 267).

Isolation. The leaves of *S. parviflorus* from the first collection were separated from the stems, air dried and extracted in a Soxhlet apparatus (Me_2CO) for 4 days. The soln was decolourized with activated charcoal, filtered through a Celite pad and the remaining Me_2CO removed *in vacuo* to give a tan coloured brittle solid (43.8 g). This solid was chromatographed on silica gel (Merck No. 7734) (250 g) and eluted with hexane–EtOAc. The frs eluted with hexane–EtOAc (1:1) gave crude oleanolic acid (1.3 g) as the major component. Flash CC of one of the frs (1.9 g), eluted with EtOAc, on silica gel (Merck No. 9385) (90 g), eluting with EtOAc–hexane (2:1), gave several frs containing α-pyrones as indicated by ^{1}H NMR. These frs were comb. to give a yellow oil (0.9 g) which gave a single spot on TLC. Acetylation of a portion of this oil (300 mg) with Ac_2O (3 ml) and pyridine (5 ml) at room temp. (16 hr) followed by the usual work up gave a pale yellow oil (360 mg) which crystallized from Et_2O–hexane to yield low melting white needles (113 mg). GC analysis (oven temp. 250°, injector temp. 250°) of these needles gave two peaks at R_t 16.0 and 21.0 mins. with a peak area ratio of 1:7.3 while further GC analysis (under the same conditions) a month later, afforded three peaks at R_t 16.0, 20.0 and 21.0 mins with a peak area ratio of 1:2.4:4.9. Attempts to separate both the unacetylated and acetylated α-pyrone mixture by flash CC were unsuccessful. However normal phase semi-prep. HPLC (4:1 EtOAc–hexane) of the unacetylated oil gave **1** (112 mg) and **2** (21 mg).

Dried leaves (60 g) from the second collection of *S. parviflorus* were treated with Me_2CO as before and the extract first column and then flash chromatographed to afford crude crystalline synparvolide C (0.78 g), which on recrystallization from EtOAc–hexane (2:1) afforded white needles of **3** (0.65 g).

Compound **1**. $[\alpha]_D^{25}$ − 31° ($CHCl_3$; *c* 1.2); UV λ_{max}^{MeOH} nm: 207 (log ε 3.75); IR ν_{max}^{NaCl} cm^{-1}: 3440 (OH), 1745 (ester carbonyl), 1710 (sh, αβ-unsatd δ-lactone), 1360, 1220, 1020, 795; EIMS (70 eV) *m/z* (rel. int.): No $[M]^+$, 267

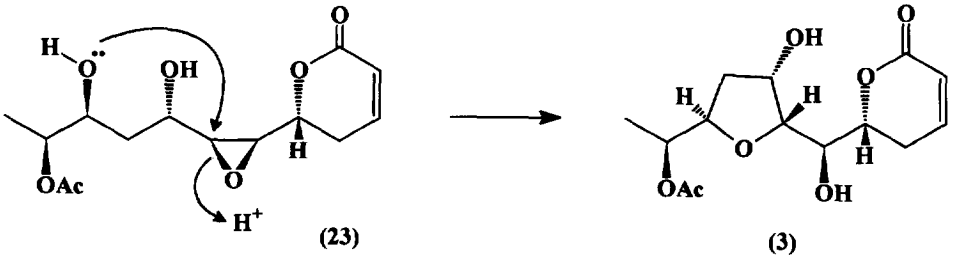


Fig. 3. Proposed biosynthesis of compound **3**.

(2), 222 (3), 196 (6), 177 (41), 140 (42), 125 (52), 97 (40), 68 (20), 43 (100). CIMS (NH_3) *m/z*: 360 $[M + 1 + NH3]^+$; ^1H NMR (400 MHz, $CDCl_3$): δ1.19 (3H, *d*, $J_{6',7'}$ = 6.5 Hz, 3H-7'), 1.88 (2H, *m*, 2H-4'), 2.03 (3H, *s*, acetyl), 2.04 (3H, *s*, acetyl), 2.59 (2H, *m*, H-5_a, H-5_e ring), 3.00 (1H, *dd*, $J_{2',3'}$ = 7.3 Hz, $J_{1',2'}$ = 4 Hz, H-2'), 3.19 (1H, *dd*, $J_{1',2'}$ = 4 Hz, $J_{1',6'}$ = 8 Hz, H-1'), 3.23 (1H, *br s*, OH), 3.59 (1H, *m*, H-3'), 4.45 (1H, *ddd*, $J_{1',6}$ = 6.9 Hz, $J_{6,5ae}$ = 8 Hz, H-6), 5.02 (1H, *dq*, $J_{5',6'}$ = 4.4 Hz, $J_{6',7'}$ = 6.5 Hz, H-6'), 5.18 (1H, *dt*, $J_{5'6'}$ = 4.5 Hz, $J_{4'5'}$ = 9.4 Hz, H-5'), 5.99 (1H, *ddd*, $J_{3,4}$ = 10.9 Hz, $J_{3,5ae}$ = 1.8 Hz, H-3), 6.90 (1H, *ddd*, $J_{3,4}$ = 10.9 Hz, $J_{4,5e}$ = 5.7 Hz, $J_{4,5a}$ = 4.3 Hz, H-4). CD (MeOH) λ_{max} = 255 nm ($\Delta\varepsilon$ = + 2.37).

Compound **2**. $[\alpha]_D^{25}$ − 11° ($CHCl_3$; *c* 1.0); UV λ_{max}^{MeOH} nm: 205 (log ε 3.82); IR ν_{max}^{NaCl} cm^{-1}: 3440 (OH), 1730 (ester carbonyl), 1710 (sh) (αβ unsaturated-δ-lactone) 1370, 1220, 1025, 950, 880; EIMS (70 eV) *m/z* (rel. int.): $[M]^+$, 326 (0.1), 206 (8), 153 (17), 135 (34), 114 (43), 97 (23), 68 (22), 43 (100); CIMS (NH_3) *m/z*: 343 $[M + NH_3]^+$; ^1H NMR (400 MHz, $CDCl_3$): δ1.20 (3H, *d*, $J_{6',7'}$ = 6.5 Hz, H-7'), 1.71 (2H, *m*, 2H-4'), 2.04 (3H, *s*, acetyl), 2.09 (3H, *s*, acetyl), 2.41 (2H, *m*, H-5_a, H-5_e ring), 2.91 (1H, *br s*, OH), 4.37 (1H, *m*, H-3'), 4.98 (1H, *dq*, $J_{6',7'}$ = 6.5 Hz, $J_{5',6'}$ = 4.8 Hz, H-6'), 5.08 (1H, *m*, H-5'), 5.36 (1H, *ddd*, $J_{1',6}$ = 6.6 Hz, $J_{5a,6}$ = 9 Hz, H-6), 5.55 (1H, *dd*, $J_{1',2'}$ = 11 Hz, $J_{2',3'}$ = 6.9 Hz, H-2'), 5.61 (1H, *dd*, $J_{1',2'}$ = 11 Hz, $J_{1',6}$ = 6.7 Hz, H-1'), 6.01 (1H, *ddd*, $J_{3,4}$ = 9.9 Hz, $J_{3,5}$ = 1.7 Hz, H-3), 6.86 (1H, *ddd*, $J_{3,4}$ = 9.9 Hz, $J_{4,5e}$ = 5.1 Hz, $J_{4,5a}$ = 3.0 Hz, H-4). CD (MeOH), λ_{max} = 255 nm ($\Delta\varepsilon$ = + 2.37). HREIMS, Obsd *m/z* = 326.1340, $C_{16}H_{22}O_7$ requires 326.1365.

Compound **3**. Mp 115–115.5°. $[\alpha]_D^{19}$ + 17° ($CHCl_3$; *c* 1.4); UV λ_{max}^{MeOH} nm: 210 nm (log ε 3.90); IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1710 (ester carbonyl), 1690 (sh), αβ-unsaturated δ-lactone), 1370, 1260, 1080, 810; EIMS (70 eV) *m/z* (rel. int.): $[M]^+$ 300 (7), 203 (32), 177 (28), 143 (43), 127 (52), 113 (45), 97 (77), 83 (100), 69 (44); ^1H NMR (400 MHz, $CDCl_3$): δ1.21 (3H, *d*, $J_{6'7'}$ = 6.4 Hz, H-7'), 1.86 (1H, *m*, H-4'$_e$), 2.08 (3H, *s*, acetyl), 2.25 (1H, *m*, H-4'$_a$), 2.47 (1H, *m*, H-5_a ring), 2.69 (1H, *m*, H-5_e ring), 3.95 (1H, *m*, H-5'), 3.99 (1H, *m*, H-1'), 4.11 (1H, *br s*, OH), 4.14 (1H, *br s*, OH), 4.20 (1H, *dd*, $J_{1',2'}$ = 4.3 Hz, $J_{2',3'}$ = 1.1 Hz, H-2'), 4.35 (1H, *ddd*, $J_{1',6}$ = 4.3 Hz, $J_{5e,6}$ = 4.2 Hz, $J_{5a,6}$ = 9.4 Hz, H-6), 4.47 (1H, *m*, H-3'), 5.16 (1H, *dq*, $J_{5'6'}$ = 7.8 Hz, $J_{6'7'}$ = 6.4 Hz, H-6'), 6.01 (1H, *ddd*, $J_{3,4}$ = 10 Hz, $J_{3,5}$ = 1.6 Hz, H-3), 6.93 (1H, *ddd*, $J_{3,4}$ = 10 Hz, $J_{4,5e}$ = 5.1 Hz, $J_{4,5a}$ = 3.0 Hz, H-4). CD (MeOH) λ_{max} 256 nm ($\Delta\varepsilon$ = + 3.4). Found: C, 56.31; H, 6.91. $C_{14}H_{20}O_7$ requires C, 55.99; H, 6.71%.

Acetylation of compound **1**. Ac_2O-pyridine treatment of **1** for 2 days at room temp. followed by normal phase, semi-prep. HPLC (EtOAc–hexane 3:1) gave **6** as an oil, $[\alpha]_D^{27}$ − 18° ($CHCl_3$; *c* 0.7). IR ν_{max}^{NaCl} cm^{-1}: 1745, 1710 (sh), 1360, 1220, 1020, 795; EIMS (70 eV) *m/z* (rel. int.): [No M]$^+$, 332 (1), 287 (12), 211 (9), 177 (17), 125 (26), 97 (24), 83 (26), 68 (13), 43 (100); CIMS (NH_3) *m/z*: 402 $[M + 1 + NH_3]^+$; ^1H NMR (400 MHz, $CDCl_3$): δ1.18 (3H, *d*, $J_{6',7'}$ = 6.5 Hz, H-7'), 2.01 (2H, *m*, H-4'), 2.04 (3H, *s*, acetyl), 2.06 (3H, *s*, acetyl), 2.08 (3H, *s*, acetyl), 2.55 (2H, *m*, H-5_a, H-5_e ring), 3.11 (1H, *dd*, $J_{1',2'}$ = 4 Hz, $J_{2',3'}$ = 8 Hz, H-2'), 3.17 (1H, *dd*, $J_{1',2'}$ = 4 Hz, $J_{1',6}$ = 8 Hz, H-1'), 4.39 (1H, *dd*, $J_{5,6}$ = 11 Hz, $J_{1'6}$ = 8 Hz, H-6), 4.77 (1H, *m*, H-3'), 4.99 (1H, *dq*, $J_{6'7'}$ = 6.5 Hz, $J_{5',6'}$ = 4.5 Hz, H-6'), 5.09 (1H, *dt*, $J_{5',6'}$ = 4.1 Hz, $J_{4',5'}$ = 10.2 Hz, H-5'), 6.02 (1H, *ddd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 1.7 Hz, H-3), 6.88 (1H, *ddd*, $J_{3,4}$ = 9.8 Hz, $J_{4,5a}$ = 4.3 Hz, $J_{4,5e}$ = 5.4 Hz, H-4).

Acetylation of compound **2**. Ac_2O-pyridine treatment of **2** for 2 days at room temp. followed by normal phase semi-prep. HPLC (EtOAc–hexane 2: 1) gave **7** as a pale yellow oil, $[\alpha]_D^{27}$ + 27° ($CHCl_3$; *c* 0.6). IR ν_{max}^{NaCl} cm^{-1}: 1740, 1720(sh), 1370, 1240, 1040, 950, 820; EIMS (70 eV) *m/z* (rel. int.) : [No M]$^+$, 281 (3), 239 (17), 206 (15), 187 (15), 161 (11), 133 (16), 97 (13), 68 (33), 43 (100); CIMS (NH_3) *m/z*: 386 $[M + 1 + NH_3]^+$.

Acetylation of compound **3**. Ac_2O-pyridine treatment of **3** for 3 days at room temp. gave **14** as a yellow oil, $[\alpha]_D^{17}$ + 26° ($CHCl_3$; *c* 1.8). ^1H NMR (400 MHz, $CDCl_3$): δ1.17 (3H, *d*, $J_{6',7'}$ = 6.5 Hz, H-7,'), 1.69 (1H, *m*, H-4'$_e$), 1.99 (3H, *s*, acetyl), 2.04 (3H, *s*, acetyl), 2.11 (3H, *s*, acetyl), 2.36 (3H, *m*, H-4'$_a$, H-5_a, H-5_e, ring), 3.92 (1H, *m*, H-5'), 4.49 (1H, *dd*, $J_{1',2'}$ = 7.3 Hz, $J_{2',3'}$ = 2.1 Hz, H-2'), 4.60 (1H, *ddd*, $J_{1',6}$ = 6.0 Hz, $J_{5a,6}$ = 4.2 Hz, $J_{5e,6}$ = 8.2 Hz, H-6), 5.03 (1H, *dq*, $J_{5',6'}$ = 7.6 Hz, $J_{6',7'}$ = 6.5 Hz, H-6'), 5.16 (1H, *dd*, $J_{1',6}$ = 6.0 Hz, $J_{1',2'}$ = 2.1 Hz, H-1'), 5.37 (1H, *q*, $J_{2',3'}$ = $J_{3',4'}$ = 7.6 Hz, H-3'), 6.00 (1H, *dd*, $J_{3,4}$ = 10 Hz, $J_{3,5}$ = 1.6 Hz, H-3), 6.85 (1H, *ddd*, $J_{3,4}$ = 10 Hz, $J_{4,5e}$ = 5.1 Hz, $J_{4,5a}$ = 3.0 Hz, H-4).

Compound **8**. Compound **6** (30 mg) was allowed to stand in air for two months. Semi-prep. HPLC on a normal phase column (EtOAc) of the resulting mixture of products gave unchanged **6** (4 mg) and **8** (14 mg) as an oil, $[\alpha]_D^{25}$ + 1° ($CHCl_3$; *c* 1.4). IR ν_{max}^{NaCl} cm^{-1}: 3480, 1750, 1710 (sh), 1390, 1250, 1040, 830; EIMS (70 eV) *m/z* (rel. int.): [No M]$^+$, 287 (1), 245 (6), 215 (10), 177 (13), 113 (17), 97 (26), 83 (41), 68 (10), 43 (100); CIMS (NH_3) *m/z*: 420 $[M + 1 + NH_3]^+$; ^1H NMR (400 MHz, $CDCl_3$): δ1.18 (3H, *d*, $J_{6',7'}$ = 6.5 Hz, 3H-7'), 1.95 (2H, *m*, 2H-4'), 2.05 (6H, *s*, 2 × acetyl), 2.07 (3H, *s*, acetyl), 2.52 (2H, *m*, H-5_a, H-5_e ring), 2.87 (1H, *s*, OH), 2.98 (1H, *s*, OH), 3.81 (1H, *m*, H-1'), 3.87 (1H, *m*, H-2'), 4.45 (1H, *ddd*, $J_{5a,6}$ = 11.2 Hz, $J_{5e,6}$ = 4.5 Hz, $J_{6,1'}$ = 6.9 Hz, H-6), 4.94 (1H, *dq*, $J_{6',7'}$ = 6.5 Hz, $J_{5',6'}$ = 4.5 Hz, H-6'), 5.02 (1H, *m*, H-5'), 5.06 (1H, *m*, H-3'), 6.0 (1H, *ddd*, $J_{3,4}$ = 9.7 Hz, $J_{3,5}$ = 1.8 Hz, H-3), 6.93 (1H, *ddd*, $J_{4,3}$ = 9.7 Hz, $J_{4,5e}$ = 6.1 Hz, $J_{4,5a}$ = 2.6 Hz, H-4).

Acetylation of compound **8**. Ac_2O-pyridine treatment of **8** for 2 days at room temp. gave **9** as a yellow oil, $[\alpha]_D^{20}$ + 5° ($CHCl_3$; *c* 1.6). ^1H NMR (400 MHz, $CDCl_3$): δ1.16 (3H, *d*, $J_{6',7'}$ = 6.3 Hz, H-7'), 1.83 (2H, *m*, 2H-4'), 2.03 (3H, *s*, acetyl), 2.04 (3H, *s*, acetyl), 2.06 (3H, *s*, acetyl), 2.08 (3H, *s*, acetyl), 2.13 (3H, *s*, acetyl), 2.39 (2H, *m*, H-5_a, H-5_e ring), 4.51 (1H, *ddd*, $J_{1',6}$ = 6.9 Hz, $J_{5a,6}$ = 8.7 Hz, $J_{5e,6}$ = 4.3 Hz, H-6), 4.93 (1H, *m*, H-5'), 4.97 (1H, *m*, H-6'), 5.09 (1H, *td*, $J_{2',3'}$ = 5.9 Hz, $J_{3',4'}$ = 8.6 Hz, H-3'), 5.24 (1H, *dd*, $J_{1',6}$ = 6.9 Hz, $J_{1',2'}$ = 3.5 Hz, H-1'), 5.31 (1H, *dd*, $H_{1',2'}$ = 3.5 Hz, $H_{2',3'}$ = 5.9 Hz, H-2'), 6.01 (1H, *dd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 2.2 Hz, H-3), 6.85 (1H, *ddd*, $J_{3,4}$ = 9.8 Hz, $J_{4,5e}$ = 5.0 Hz, $J_{4,5a}$ = 3.1 Hz, H-4).

Isopropylidene derivative of compound **3**. A soln of **3** (90 mg) in Me_2Co (3 ml) was left on a column (1 cm × 10 cm) of Amberlyst 15 for 5 min and then slowly eluted with Me_2Co. The solvent was evaporated, the product dissolved in EtOAc and passed through a short column of alumina and crystallized from EtOAc–hexane (1: 4) to afford white needles (68 mg) of **15**, mp 130–131°, $[\alpha]_D^{21}$ + 34 ($CHCl_3$; *c* 1.1). IR ν_{max}^{KBr} cm^{-1}: 1730, 1385, 1260, 1095, 955, 815, 795; ^1H NMR (400 MHz, $CDCl_3$): δ1.16 (3H, *d*, $J_{6',7'}$ = 6.4 Hz, H-7'), 1.38 (3H, *s*, acetonide Me), 1.40 (3H, *s*, acetonide Me), 1.82 (1H, *m*, H-4'$_e$), 2.02 (3H, *s*, acetyl), 2.18 (1H, *m*, H-4'$_a$), 2.45 (1H, *m*, H-5$_e$ ring), 2.60 (1H, *m*, H-5$_a$ ring), 3.71 (1H, *dd*, $J_{1',2'} = J_{2',3'}$ = 2.3 Hz, H-2'), 3.99 (1H, *m*, H-5'), 4.08 (1H, *dd*, $J_{1',6}$ = 6.6 Hz, $J_{1',2'}$ = 2.3 Hz, H-1'), 4.35 (1H, *dd*, $J_{2',3'}$ = 2.3 Hz, $J_{3',4'}$ = 6.1 Hz, H-3'), 4.67 (1H, *ddd*, $J_{1',6}$ = 6.6 Hz, $J_{5a,6}$ = 9.3 Hz, $J_{5e,6}$ = 4.1 Hz, H-6), 5.14 (1H, *m*, H-6'), 5.98 (1H, *dd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 1.5 Hz, H-3), 6.87 (1H, *ddd*, $J_{3,4}$ = 9.8 Hz, $J_{4,5e}$ = 5.0 Hz, $J_{4,5a}$ = 3.1 Hz, H-4). Found: C, 60.33; H, 6.93. $C_{17}H_{24}O_7$ requires C, 59.99; H, 7.11.

(S)- *and* (R)-*MTPA esters of compound* **1**. A soln of **1** (5 mg) in CH_2Cl_2 (1 ml) was treated at room temp. with (R)-MTPA (22 mg), DCC (90 mg) and DMAP (7 mg) for 30 min. The soln was diluted with water and EtOAc, worked up as usual and chromatographed on silica gel in benzene–hexane–EtOAc. The fr. eluted with EtOAc gave the (*R*)-MTPA ester as needles (8 mg). The (*S*)-MTPA ester was prepared similarly.

Isopropylidine derivative of saponified compound **3**. Compound **3** (40 mg) was stirred 24 h with 7 ml of 0.12 M NaOH, the soln acidified with 1.5 ml 1.5 M HCl and heated 2 min on the steambath to complete lactonization of the free acid. The cooled soln was saturated by the addition of NaCl (3 g) to facilitate extraction of the very water soluble product with Et_2O in an efficient extraction apparatus (7 hr). The product (23 mg) was converted to the acetonide as before to afford white needles (18 mg), from EtOAc–hexane, mp 130–130.5°, $[\alpha]_D^{22}$ + 44° ($CHCl_3$; *c* 0.9). ^1H NMR (400 MHz, $CDCl_3$) δ 1.17 (3H, *d*, $J_{6',7'}$ = 6.4 Hz, H-7'), 1.37 (3H, *s*, acetonide Me), 1.44 (3H, *s*, acetonide Me), 1.89 (1H, *m*, H-4'$_e$), 2.28 (1H, *m*, H-4'$_a$), 2.45 (1H, *m*, H-5$_e$ ring), 2.67 (1H, *m*, H-5$_a$ ring), 3.79 (2H, *m*, H-2', H-6'), 3.95 (1H, *m*, H-5'), 4.10 (1H, *dd*, $J_{1,6}$ = 7.3 Hz, $J_{1',2'}$ = 2.0 Hz, H-1'), 4.38 (1H, *dd*, $J_{2',3'}$ = 2.1 Hz, $J_{3',4'}$ = 5.0 Hz, H-3'), 4.74 (1H, *ddd*, $J_{1',6}$ = 7.3 Hz, $J_{5a,6}$ = 9.3 Hz, $J_{5e,6}$ = 4.1 Hz, H-6), 6.01 (1H, *dd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 2.4 Hz, H-3), 6.91 (1H, *ddd*, $J_{3,4}$ = 9.8 Hz, $J_{4,5e}$ = 5.0 Hz, $J_{4,5a}$ = 3.1 Hz, H-4). Found: C, 60.05; H, 6.96. $C_{15}H_{22}O_6$ requires C, 60.4; H, 7.4%.

Acknowledgements—We thank Mr. T. J. Edwards of the Botany Department, University of Natal, Pietermaritzburg for the collection and identification of the plant material, Professor Ferreira of the University of the Orange Free State for the CD measurements, Dr Pereda-Miranda and Professor Kjaer for samples of hyptolide and 5-deacetoxy-5'-epi-olguine and Rhodes University and the Foundation for Research Development for financial assistance.

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