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A NEW CLASS OF MACROCYCLIC DITERPENES FROM BERTYA DIMEROSTIGMA (EUPHORBIACEAE)

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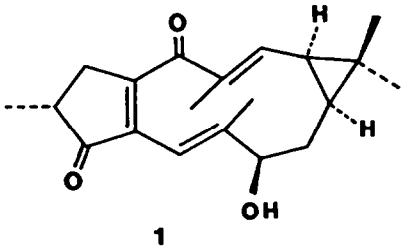
Abstract - Three diterpene acids, representing a new class of bicyclic diterpenes, have been isolated from Bertya dimerostigma F. Muell. (Euphorbiaceae). Spectroscopic and X-ray crystallographic evidence for their structure is presented. A rationale for their origin via modification of the casbane skeleton is given.

Previous work[1] on the constituents of Bertya cupressoidea (Ricinocarpoideae, Euphorbiaceae) had shown the presence of a number of macrocyclic diterpenes of the lathyrane class, e.g. (1), which together with a number of toxic diterpenes from the Euphorbiaceae are considered to arise by elaboration of the casbene skeleton.[2] An examination of the metabolites from B. dimerostigma has revealed the presence of members of a new class of macrocyclic diterpenes which co-occur with lathyrane diterpenes. Spectroscopic and X-ray crystallographic evidence for the structure of three new macrocyclic diterpene acids (2, 3 and 4) is presented in this report and a rationale for their origin is given. These compounds incorporate a new macrocyclic skeleton for which the trivial name berdimerane has been adopted together with the numbering system shown in (5).

The major components of the acidic fraction from an extract of B. dimerostigma were separated after treatment with ethereal diazomethane and preparative radial chromatography of the derived methyl esters. Three compounds were isolated and designated methyl esters C(2), B(3) and A(4). The major compounds B and C were crystalline whereas compound A was obtained as a colourless and unstable oil. All three compounds had molecular formula $C_{21}H_{32}O_4$ requiring six double bond equivalents. The infrared spectra of each showed absorptions for three carbonyl functions corresponding to a methyl ester and two ketones. The ^{13}C-NMR spectra confirmed this showing signals for two carbons in the region δ 207-212 and for one carbon at δ 175-176. The presence of a trisubstituted double bond was inferred from signals for sp^2-hybridised carbons at ∿ δ 140 (s) and ∿ δ 118 (d). Other resonances in the ^{13}C-NMR spectra indicated, in each case, one quaternary, five methine, four methylene and six methyl carbons. The ^{1}H-NMR spectra included signals for an olefinic methyl, two secondary and two tertiary methyl groups. Signals between δ 0.5-0.0 (2H) suggested the presence of cyclopropyl protons and since the compounds are bicyclic a system containing a macrocyclic ring fused to a cyclopropane, reminiscent of the casbane skeleton, was considered as a possibility.

Structures of methyl esters C(2) and A(4)

The ^{1}H-NMR spectrum of methyl ester C(2) taken at 300 MHz still showed a number of overlapping spin patterns consequently proton connectivity sequences were determined by homonuclear correlated 2D-techniques (COSY) and coupling constants were obtained by extensive NMDR and homonuclear J resolved 2D-measurements. The results are summarised in Fig. 1 in terms of the partial



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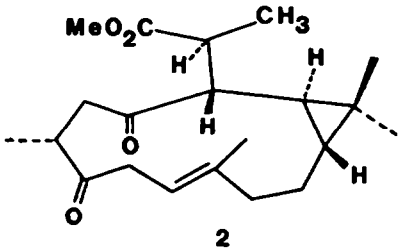

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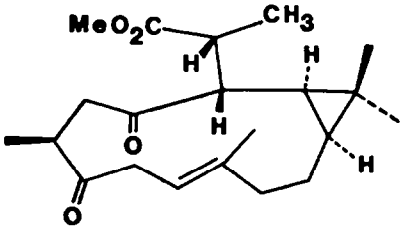

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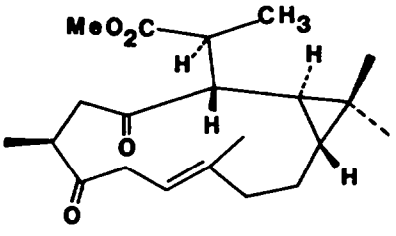

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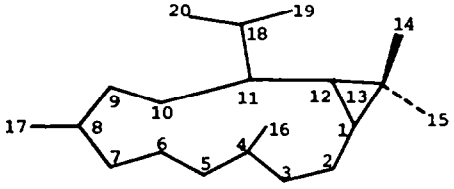

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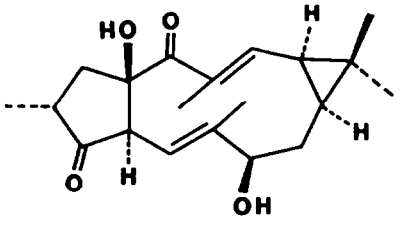

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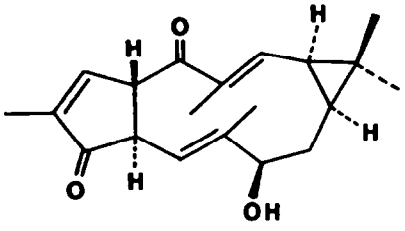

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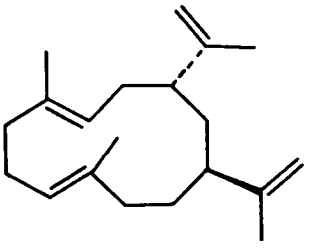

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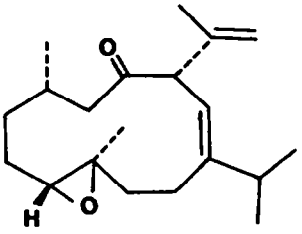

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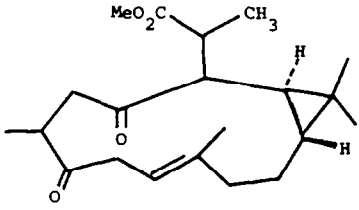
structures (I), (II) and (III) which could be recognised. In partial structure (I) the 1,4-diketone moiety is implied by the large coupling constants of the geminal α-protons and by the chemical shift of the α-methine proton. Furthermore the carbonyl group in substructure (II) corresponds to one of those in substructure (I). The E-configuration of the double bond in (II) is inferred from the chemical shift of the carbon of the olefinic methyl which resonates at δ 15.0. Substructure(III) includes a trans-disubstituted cyclopropane ring since the cyclopropyl methine protons show mutual coupling of J 5.5 Hz.[3] In addition the methyl ester functionality must be placed α- or β- to the secondary methyl group in substructure (III) although a distinction cannot be made on spectroscopic grounds. A number of possible structures can be generated by combination of substructures (I) to (III) but these can be reduced on consideration of two points. Firstly, the absence of absorption at λ >215 nm in the U.V. spectrum of (2) excludes the linkage of the olefinic carbon of substructure II to a carbonyl carbon of substructure I. Secondly, each of the substructures include isoprenoid fragments and if the assumption is made that methyl ester C is derived biogenetically from a polyisoprenoid then two "best-fit" structures (IV and V) can be assembled.



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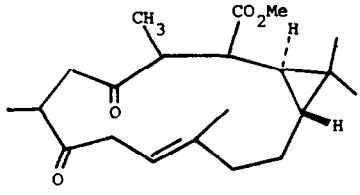


V

Since chemical or spectroscopic distinction between IV and V seemed inordinately difficult an answer to this problem was sought through single-crystal X-ray analysis. The results, outlined below, point in favour of structure IV and thus the structure and relative stereochemistry of methyl ester C is as designated in (2).

Treatment of methyl ester C(2) with a mixture of pyridine-water at 104° generated a two component mixture which could be separated into (2) (60%) and another compound (40%) identical with methyl ester A which has been assigned structure (4) on the following evidence. The ^{13}C-NMR spectrum of (4) corresponded closely to that of (2) for all carbons except those assigned to C8, C9 and C17 (Table 1). The ^{1}H-NMR spectrum of (4) showed the downfield proton at C9 to have a small coupling (J 3.7 Hz) to the vicinal methine proton in contrast to the situation observed in the spectrum of (2). In addition the proton at C8 had been deshielded by 0.65 ppm. All these points support the argument that methyl ester A is the C8 epimer of (2).

Interestingly, treatment of (2) with d_5 pyridine-$^{2}H_2O$ under similar conditions yielded mainly the 6-d_1 analogue of (2) and the 6,6',8-d_3 analogue of (4). Furthermore treatment of (2) or (4) under basic or acidic conditions (p-toluenesulphonic acid in refluxing dichloromethane) leads to a mixture of (2) and (4) in a ratio of 60:40.

Structure of methyl ester B (3)

Analysis of the COSY spectrum obtained for methyl ester B (3) in conjunction with NMDR and J-resolved 2D-^{1}H-NMR experiments indicated that substructures I, II and III were present. However significant differences in chemical shift and coupling constants of several protons were observed when the ^{1}H-NMR spectrum of (3) was compared with that of (2) indicating their diastereomeric relationship. In particular, the cyclopropyl protons of (3) showed a mutual coupling of J 8.7 Hz suggesting,[3] but not exclusively, a cis-fusion of the cyclopropane to the macrocyclic ring. A distinction between the possible diastereoisomers of (2) and (4) was not possible and again the isomeric structure (V) could not be excluded. Consequently an X-ray crystallographic study of methyl ester B was undertaken and the results described below show the structure and relative stereochemistry of the compound to be as shown in (3) differing from (2) in the configuration at

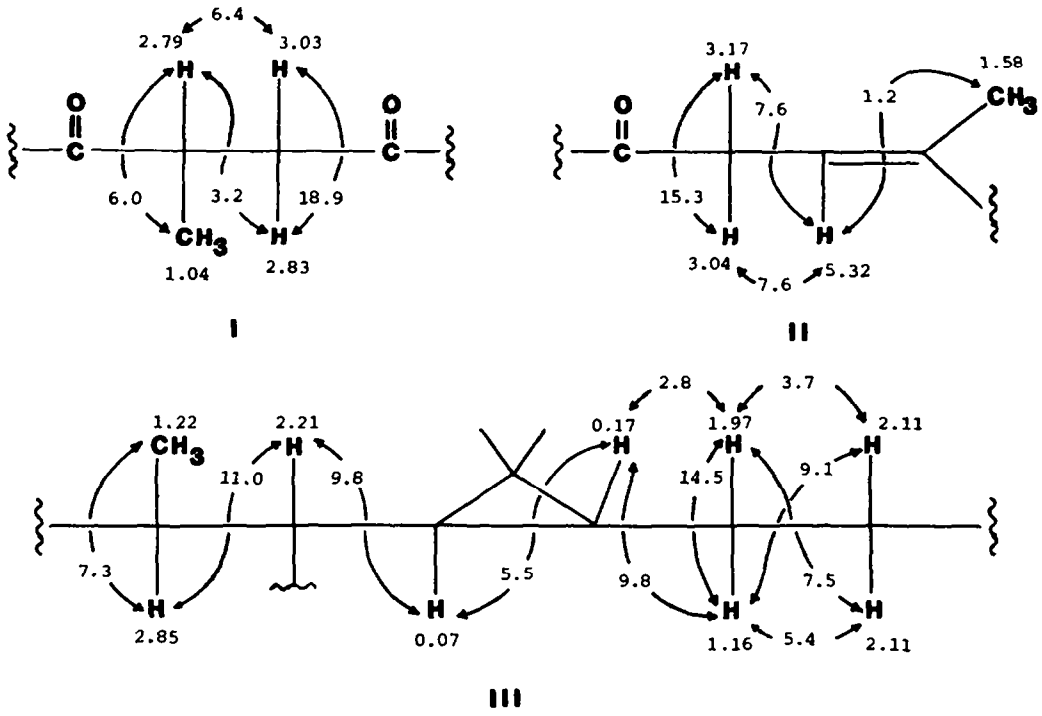


Figure 1 Chemical shifts (δ) and coupling constants (Hz) derived from ^{1}H-NMDR measurements of methyl ester C (2)

Table 1. ^{13}C-NMR spectra of (2), (3) and (4). Chemical shifts (δ) in ppm relative to TMS[a].

Carbon	2[b]	4[c]	3[b]	Carbon	2[b]	4[c]	3[b]
1	27.2	26.3	27.8	12	32.5	32.6	27.0
2	25.4	25.2	24.0	13	19.9	20.0	17.5
3	39.3	39.3	39.6	14	23.0[e]	23.2[e]	15.4
4	137.8	138.4	140.0	15	21.5[e]	21.5[e]	29.0
5	118.7	118.5	118.4	16	15.0	14.9	18.2
6	43.2	43.9	43.3	17	15.5	16.2	16.5
7	208.7[d]	209.9[d]	207.8[d]	18	42.8	42.9	42.7
8	41.1	38.2	40.5	19	14.9	14.9	13.0
9	45.6	49.5	47.7	20	175.8	176.3	175.1
10	210.3[d]	212.3[d]	211.4[d]	OCH_3	51.8	51.8	52.0
11	53.2	51.7	49.6				

a Multiplicities of signals were determined by SFORD and GASPE techniques and are consistent with assignments

b Spectrum obtained at 75.46 MHz

c Spectrum obtained at 20.1 MHz

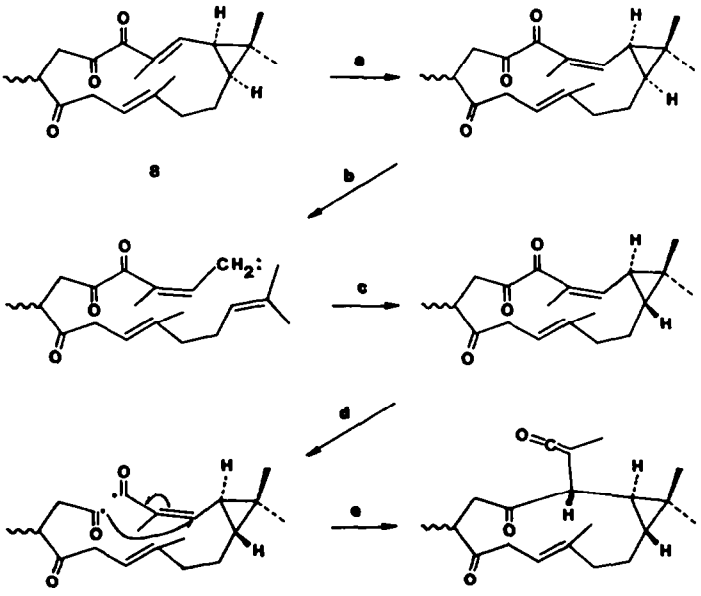
d-e Values in any one column may be interchanged.

C1, C8 and C18.

Thus the three metabolites from Bertya dimerostigma described in this report have been shown to represent a new class of bicarbocyclic diterpenes. Since these metabolites co-occur with the known[1] lathyrane compounds (1), (6) and (7) (see Experimental) it is tempting to speculate that both classes of diterpenes, lathyranes and berdimeranes, arise from a common casbene precursor e.g. (8). Trans-annular aldol condensation of (9) would lead to the lathyrane skeleton represented by (1), (6) and (7). It is worth noting however, that the berdimeranes occur in a surface resin from an area of very high light intensity. Furthermore, resins from similar plants have been shown, in some cases,[4,5] to contain compounds which can also be generated photochemically from a co-occurring metabolite. With this in mind one possible explanation for the formation of the berdimerane skeleton is that it might arise from the likely precursor (8) by a series of photolytic type processes as shown in Scheme 1. The initial steps which involve E- to Z-isomerisation of the double bond (step a), fragmentation of the cyclopropane ring, rotation of the terminal olefin and recyclization to give the trans-ring fused cyclopropane (steps b and c) have precedence in the photochemical behaviour of 6 and similar compounds.[5] Subsequent cleavage of the 1,2-diketone (step d) followed by C-C bond formation between the carbonyl radical and the β-carbon of the α,β-unsaturated carbonyl (step e) results overall in the extrusion of an isopropyl ketene moiety which on hydration would lead to the berdimerane acid corresponding to methyl ester C (2). The formation of the acid corresponding to methyl ester B (3) can be understood in terms of cleavage of the 1,2-diketone competing favourably with cyclopropane ring fragmentation before, more probably after E- to Z-isomerisation of the double bond.

From the point of view of stereochemistry some support for this hypothesis could be obtained if the absolute stereochemistry of compounds (2) and (3) were known. Work towards this end is in progress.

In passing, it is worth noting that similar 12-membered ring monocyclic ring systems have been identified in compounds isolated from defensive secretions of termites[6] e.g. cubitene (9) and from the sea whip,[7] e.g. compound (10). For the latter, a photochemical-like rearrangement of a β,γ-unsaturated ketone can also be considered.



Scheme I : Possible genesis of the berdimanes

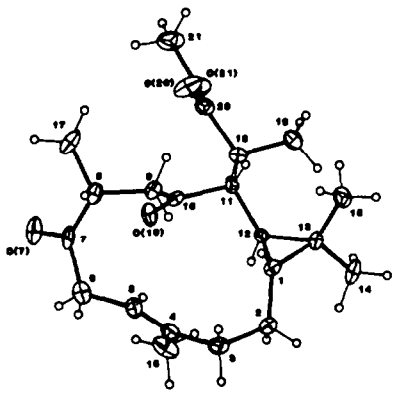
CRYSTALLOGRAPHY

Unique data sets were measured at 295 K using Syntex $P\bar{1}$ and $P\bar{2}_1$ four-circle diffractometers in conventional $2\theta/\theta$ scan mode. Graphite-monochromated Mo $K\alpha$ radiation sources were used ($\lambda = 0.7106_9$ Å). N independent reflections were measured within a $2\theta_{max}$ limit determined by the scope of the data; N_o with $I>3\sigma(I)$ were considered 'observed' and used in the (basically) 9 x 9 block diagonal least squares refinement after solution of the structures by direct methods without absorption connection. Anisotropic thermal parameters were refined for the non-hydrogen atoms; $(x, y, z, U_{iso})_H$ were constrained at estimated values. At convergence R, R' on $|F|$ are quoted. Reflections weights were $(\sigma^2(F_o) + 0.0005(F_o)^2)^{-1}$. Neutral complex scattering factors were used;[9] computation used the XTAL 83 program system implemented by S.R. Hall on a Perkin-Elmer 3240 computer.[10] Material deposited comprises thermal and hydrogen parameters, structure factor amplitudes.

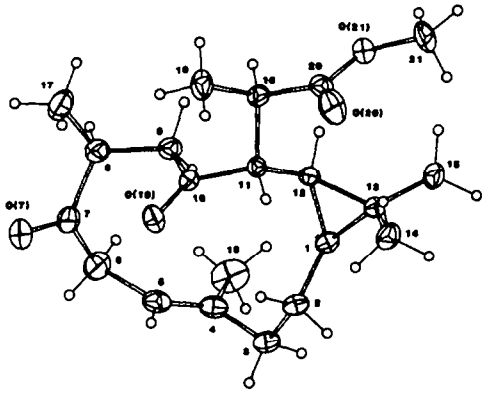
Crystal data. (2) $C_{21}H_{32}O_4$, M = 348.5, Monoclinic, space group $P2_1$ (C_2^2, No.4), a = 10.280-(4), b = 10.752(3), c = 10.196(4) Å, β = 114.78(3)°, U = 1023.3(6) Å^3. D_m = 1.12(1), D_c (Z = 2) = 1.13 g.cm.$^{-3}$ $F(000)$ = 380. μ_{Mo} = 0.82 cm.$^{-1}$ Specimen: 0.50 x 0.21 x 0.16 mm. $2\theta_{max}$ = 50°. N = 1920, N_o = 1208. R = 0.052, R' = 0.043.

(3) $C_{21}H_{32}O_4$, M = 348.5, Orthorhombic, space group $P2_12_12_1$ (D_2^4, No.19), a = 32.09(1), b = 10.158(4), c = 6.212(2) Å, U = 2025(1) Å^3. D_m = 1.13(1), D_c (Z = 4) = 1.14 g.cm.$^{-3}$ $F(000)$ = 760. μ_{Mo} = 0.83 cm.$^{-1}$ Specimen: 0.48 x 0.30 x 0.24 mm. $2\theta_{max}$ = 50°. N = 1850, N_o = 1235. R = 0.052, R' = 0.052.

The results of the two structure determinations are consistent with the stoichiometry and connectivities proposed above, and definitive of stereochemistry and conformation but not chirality. In each case, the asymmetric unit of the structure comprises one molecule; there are no abnormal intermolecular interactions. Bond lengths and angles are substantially as expected, although significant differences within the macrocycle are observed at C(6) and also at C(11) (Table 3).



(2)



(3)

FIGURE 2. Molecular projections of (2) and (3)
20% probability amplitude thermal ellipsoids and non-hydrogen atom labelling schemes are shown; hydrogen atoms have an arbitrary radius of 0.1Å. Close transannular hydrogen contacts are observed in (2): H(5)...H(9b), 2.2_3Å; H(12)...H(16a) 2.3_8Å.

Table 2. Non-hydrogen atom coordinates

Atom	(2) x	(2) y	(2) z	(3) x	(3) y	(3) z
C(1)	0.4038(6)	0*	0.2487(6)	0.9295(2)	0.8579(5)	1.1217(10)
C(2)	0.4865(6)	0.0140(9)	0.1578(7)	0.9561(2)	0.8743(6)	0.9246(10)
C(3)	0.3960(7)	-0.0044(9)	-0.0049(7)	0.9848(2)	0.9946(7)	0.9277(11)
C(4)	0.2800(7)	0.0882(9)	-0.0739(7)	0.9629(2)	1.1259(7)	0.9454(13)
C(5)	0.1471(7)	0.0542(9)	-0.1428(7)	0.9431(2)	1.1720(7)	0.7761(12)
C(6)	0.0184(8)	0.1328(11)	-0.2250(7)	0.9177(2)	1.2986(6)	0.7581(15)
C(7)	-0.0903(6)	0.1633(9)	-0.1683(7)	0.8750(2)	1.2795(6)	0.6690(15)
O(7)	-0.1609(5)	0.2561(7)	-0.2115(5)	0.8675(2)	1.3054(6)	0.4837(10)
C(8)	-0.1207(6)	0.0697(8)	-0.0724(7)	0.8408(2)	1.2394(6)	0.8231(12)
C(9)	0.0065(6)	0.0358(8)	0.0697(6)	0.8497(2)	1.1123(6)	0.9518(11)
C(10)	0.0973(6)	0.1433(8)	0.1527(6)	0.8576(2)	0.9946(6)	0.8150(10)
O(10)	0.0849(4)	0.2457(6)	0.1016(4)	0.8665(1)	1.0049(4)	0.6274(7)
C(11)	0.2063(5)	0.1183(7)	0.3061(5)	0.8562(2)	0.8583(5)	0.9196(9)
C(12)	0.3539(6)	0.1110(7)	0.3070(5)	0.8822(2)	0.8502(5)	1.1211(9)
C(13)	0.4720(6)	0.0251(8)	0.4079(6)	0.9082(2)	0.7301(6)	1.1789(9)
C(14)	0.6208(6)	0.0783(8)	0.4764(7)	0.9126(2)	0.6160(6)	1.0264(10)
C(15)	0.4440(7)	-0.0655(8)	0.5048(7)	0.9080(2)	0.6887(7)	1.4108(10)
C(16)	0.3248(9)	0.2208(10)	-0.0609(8)	0.9628(2)	1.1926(8)	1.1588(14)
C(17)	-0.2487(7)	0.1118(11)	-0.0458(8)	0.7984(2)	1.2329(7)	0.7174(16)
C(18)	0.1979(6)	0.2237(8)	0.4048(6)	0.8098(2)	0.8201(6)	0.9622(10)
C(19)	0.3018(6)	0.2097(8)	0.5620(6)	0.7829(2)	0.8401(7)	0.7630(12)
C(20)	0.0459(8)	0.2339(9)	0.3934(7)	0.8075(2)	0.6782(7)	1.0314(12)
O(20)	-0.0420(5)	0.1561(8)	0.3524(6)	0.8097(2)	0.5863(5)	0.9131(9)
O(21)	0.0290(6)	0.3440(7)	0.4445(6)	0.8027(1)	0.6678(4)	1.2424(7)
C(21)	-0.1063(9)	0.3654(9)	0.4484(9)	0.7991(2)	0.5354(7)	1.3271(12)

* defines origin

Table 3. Non-hydrogen interatomic distances (Å)

Atoms	(2)	(3)	Atoms	(2)	(3)
C(1)-C(2)	1.506(11)	1.501(9)	C(9)-C(10)	1.503(10)	1.489(9)
C(1)-C(12)	1.515(9)	1.519(7)	C(10)-O(10)	1.201(11)	1.204(7)
C(1)-C(13)	1.498(8)	1.510(8)	C(10)-C(11)	1.519(7)	1.531(8)
C(2)-C(3)	1.537(9)	1.530(9)	C(11)-C(12)	1.516(8)	1.508(8)
C(3)-C(4)	1.485(11)	1.511(10)	C(11)-C(18)	1.542(10)	1.561(7)
C(4)-C(5)	1.300(10)	1.314(10)	C(12)-C(13)	1.529(9)	1.520(8)
C(4)-C(16)	1.487(14)	1.489(12)	C(13)-C(14)	1.503(9)	1.504(8)
C(5)-C(6)	1.496(11)	1.527(9)	C(13)-C(15)	1.499(11)	1.501(8)
C(6)-C(7)	1.494(12)	1.491(11)	C(18)-C(19)	1.515(7)	1.521(9)
C(7)-C(8)	1.524(12)	1.511(11)	C(18)-C(20)	1.522(11)	1.506(9)
C(7)-O(7)	1.204(11)	1.205(11)	C(20)-O(20)	1.173(11)	1.190(9)
C(8)-C(9)	1.534(7)	1.545(9)	C(20)-O(21)	1.333(12)	1.324(9)
C(8)-C(17)	1.520(12)	1.513(10)	O(21)-C(21)	1.427(12)	1.448(8)

Table 4. Non-hydrogen interatomic angles (degrees)

Atoms	(2)	(3)	Atoms	(2)	(3)
C(2)-C(1)-C(12)	122.3(5)	124.8(5)	C(10)-C(11)-C(12)	108.6(5)	112.6(4)
C(2)-C(1)-C(13)	121.3(5)	123.0(5)	C(10)-C(11)-C(18)	108.9(5)	109.0(4)
C(12)-C(1)-C(13)	61.0(4)	60.3(4)	C(12)-C(11)-C(18)	111.3(5)	112.0(5)
C(1)-C(2)-C(3)	114.1(5)	114.8(5)	C(11)-C(12)-C(1)	122.7(5)	123.6(5)
C(2)-C(3)-C(4)	115.3(7)	115.3(5)	C(11)-C(12)-C(13)	123.1(6)	122.9(5)
C(3)-C(4)-C(5)	121.4(8)	118.7(7)	C(1)-C(12)-C(13)	59.0(4)	59.6(4)
C(16)-C(4)-C(3)	116.4(6)	117.9(6)	C(1)-C(13)-C(12)	60.0(3)	60.2(4)
C(16)-C(4)-C(5)	122.2(8)	123.3(7)	C(1)-C(13)-C(14)	119.7(6)	118.2(5)
C(4)-C(5)-C(6)	128.8(9)	128.0(7)	C(1)-C(13)-C(15)	117.7(6)	118.0(5)
C(5)-C(6)-C(7)	122.4(7)	114.1(5)	C(12)-C(13)-C(14)	116.7(7)	121.4(5)
C(6)-C(7)-C(8)	119.4(8)	117.8(8)	C(12)-C(13)-C(15)	121.2(5)	116.7(5)
C(6)-C(7)-O(7)	118.3(8)	120.7(7)	C(14)-C(13)-C(15)	112.3(5)	112.9(5)
C(8)-C(7)-O(7)	121.9(7)	121.2(7)	C(11)-C(18)-C(19)	114.2(6)	111.7(5)
C(7)-C(8)-C(9)	116.1(5)	114.8(5)	C(11)-C(18)-C(20)	109.9(5)	109.4(4)
C(7)-C(8)-C(17)	110.1(7)	112.9(7)	C(19)-C(18)-C(20)	109.5(6)	109.5(5)
C(9)-C(8)-C(17)	111.2(6)	110.8(5)	C(18)-C(20)-O(20)	126.1(9)	124.9(6)
C(8)-C(9)-C(10)	115.6(6)	114.0(6)	C(18)-C(20)-O(21)	109.4(7)	111.4(6)
C(9)-C(10)-C(11)	117.4(7)	118.6(5)	O(20)-C(20)-O(21)	124.4(8)	123.8(6)
C(9)-C(10)-O(10)	122.2(5)	121.6(6)	C(20)-O(21)-C(21)	116.3(7)	116.3(5)
C(11)-C(10)-O(10)	120.5(6)	119.7(5)			

EXPERIMENTAL

General experimental details have been described[8]. In addition, preparative radial chromatography was carried out using a Chromatotron Model 7924T (Harrison Research, Palo Alto, California) with 1 mm amd 4 mm layers of Kieselgel 60 PF_{254} gipshaltig (Merck Art. 7749) spread on glass plates, and under a nitrogen atmosphere. For capillary gas liquid chromatography a Hewlett Packard 5790A Series Gas Chromatograph equipped with a Hewlett Packard 3390A Integrator, using an HP 50 m ULTRA 1 (OV1) column and operating on a splitless mode was used. The carrier gas was hydrogen. High resolution ^{1}H-NMR, J-resolved 2D ^{1}H-NMR, 2D ^{1}H-NMR homonuclear COSY (correlated spectroscopy) and 2D ^{13}C/^{1}H HETCOR (heteronuclear correlated) spectra were recorded at the Brisbane NMR Centre, Griffith University, Nathan, Queensland, using a Bruker CXP-300 Spectrometer operating at 300 MHz and at the Varian Centre, Palo Alto, USA, using a Varian HX-300 Spectrometer operating at 300 MHz. Chemical shifts are measured on the δ scale, with TMS as an external standard. High resolution ^{13}C-NMR spectra were recorded using a Bruker CXP-300 Spectrometer and a Varian HX-300 Spectrometer both operating at 75.46 MHz. Chemical shifts are measured in ppm relative to 0.0 ppm.

Isolation and separation of the metabolites from B. dimerostigma. Dry leaves and terminal branches of B. dimerostigma (female) (1.51 kg) collected 87 Km east of Southern Cross, Western Australia, were extracted twice with ether to give a crude oily extract (163g). A portion (114g) of the extract was partitioned between MeOH and light petroleum and the methanolic layer was saturated with water and repeatedly extracted with CH_2Cl_2 to give a viscous oil (72.1 g). A portion (36g) of this oil was eluted with EtOAc through neutral charcoal to yield a clear red oil (25.2g) which was fractionated into $NaHCO_3$-soluble (13.3g) and neutral fractions (10.05g).

Chromatography of a portion (500 mg) of the neutral fraction on alumina (act.III, neutral, 25g) and elution with 20-60% EtOAc-CH_2Cl_2 gave bertyadionol (6) (109 mg) identical with an authentic sample. Rapid silicic acid chromatography of the remainder of the neutral fraction (9.45g) gave on elution with 10-20% EtOAc-CH_2Cl_2 the enone (7) (1.61g) identical with an authentic sample. Further elution with 45-55% EtOAc-CH_2Cl_2 yielded the hydroxydiketone (1) (1.67g) identical with an authentic sample.

The acid fraction (13.3g) was subjected to rapid silicic acid chromatography and elution with 25-30% EtOAc-CH_2Cl_2 yielded a fraction (3.22 g) which after treatment with ethereal CH_2N_2 for 2 min. appeared as a three component mixture by t.l.c. analysis. This mixture was separated by preparative radial chromatography (13% EtOAc-light petroleum) into the following compounds: (a) methyl ester A (4) (15 mg) identical with a sample prepared as described below; (b) methyl ester B (3) (671 mg) crystallised from MeOH as needles, m.p. 73-74°; $[\alpha]_D$ -12.3° (c, 3.6; $CHCl_3$), $[\alpha]_{578}$ -13.3°, $[\alpha]_{546}$ -15.0°, $[\alpha]_{436}$ -31.9°, $[\alpha]_{365}$ -76.2° ($C_{21}H_{32}O_4$ requires C, 72.4; H, 9.3%. Found: C, 72.7; H, 9.1%). Single peak on capillary *GLC*: R_t 23.52 min. (initial temp. 70° programmed at 30°/min. to 130°, then 5°/min. to 275°). ^{1}H-NMR (300 MHz; $CDCl_3$): the following parameters were obtained from extensive NMDR experiments. δ 5.11 (tq, $J_{5,6}$ 7.2 Hz, $J_{5,6'}$ 7.2 Hz, $J_{5,16}$ 1.2 Hz, H5); 3.70 (s, methoxyl protons); 3.14 (dd, $J_{6,6'}$ 13.4 Hz, $J_{5,6}$ 7.2 Hz, H6); 3.13 (ddq, $J_{8,17}$ 6.8 Hz, $J_{8,9}$ 6.6 Hz, $J_{8,9'}$ 4.7 Hz, H8); 3.03 (dd, $J_{6,6'}$ 13.4 Hz, $J_{5,6'}$ 7.2 Hz, H6'); 2.82 (dd, $J_{9,9'}$ 19.0 Hz, $J_{8,9}$ 6.6 Hz, H9); 2.66 (dq, $J_{18,19}$ 7.1 Hz, $J_{11,18}$ 5.5 Hz, H18); 2.52 (dd, $J_{11,12}$ 11.3 Hz, $J_{11,18}$ 5.5 Hz, H11); 2.48 (dd, $J_{9,9'}$ 19.0 Hz, $J_{8,9'}$ 4.7 Hz, H9'); 2.34 (ddd, $J_{3,3'}$ 12.6 Hz, $J_{2',3}$ 6.1 Hz, $J_{2,3}$ 3.3 Hz, H3); 1.89 (ddd, $J_{3,3'}$ 12.6 Hz, $J_{2,3'}$ 6.4 Hz, $J_{2',3'}$ 3.2 Hz, H3'); 1.82 (dddd, $J_{2,2'}$ 14.3 Hz, $J_{2,3'}$ 6.4 Hz, $J_{2,3}$ 3.3 Hz, $J_{1,2}$ 1.1 Hz, H2); 1.72 (d, $J_{5,16}$ 1.2 Hz, $(H16)_3$); 1.12 (d, $J_{18,19}$ 7.1 Hz, $(H19)_3$); 1.06 (s, $(H15)_3$); 1.06 (d, $J_{8,17}$ 6.8 Hz, $(H17)_3$); 1.02 (dd, $J_{11,12}$ 11.3 Hz, $J_{1,12}$ 8.7 Hz, H12); 0.94 (dddd, $J_{2,2'}$ 14.3 Hz, $J_{1,2'}$ 9.5 Hz, $J_{2',3}$ 6.1 Hz, $J_{2',3'}$ 3.2 Hz, H2'); 0.87 (s, $(H14)_3$); 0.52 (ddd, $J_{1,2'}$ 9.5 Hz, $J_{1,12}$ 8.7 Hz, $J_{1,2}$ 1.1 Hz, H1). ^{13}C-NMR: see Table 1. GC-EIMS: R_t 5.0 min. on OV-101 (0.31 mm x 25 m, WCOT capillary column, initial temp. 140°, programmed at 20°/min.): m/z 348 (M^+, 4%), 320 (5), 317 (2), 316 (2), 305 (12), 289 (3), 273 (3), 261 (14), 245 (8), 219 (13), 205 (12), 199 (13), 193 (12), 183 (11), 179 (11), 171 (36), 151 (32), 149 (15), 139 (100), 135 (32), 123 (24), 111 (6), 109 (25), 81 (25). IR: (CCl_4) λ_{max} 1740, 1710, 1450, 1430, 1400, 1375, 1350, 1190, 1170, 1160 cm^{-1}; and (c) methyl ester C (2) (347 mg) which crystallised from MeOH as needles, m.p. 105-106°, $[\alpha]_D$ +16.6° (c, 2.1; $CHCl_3$), $[\alpha]_{578}$ +17.5°, $[\alpha]_{546}$ +20.7°, $[\alpha]_{436}$ +41.3°, $[\alpha]_{365}$ +83.0°. ($C_{21}H_{32}O_4$ requires C, 72.4; H, 9.3%. Found: C, 72.6; H, 9.2%). Single peak on capillary GLC: R_t 24.32 min. (initial temp. 70° programmed at 30°/min. to 130°, then 5°/min. to 275°). ^{1}H-NMR (300 MHz; $CDCl_3$): the following parameters were obtained in conjunction with NMDR experiments and a J-resolved 2D ^{1}H-NMR spectrum. δ 5.32 (tq, $J_{5,6}$ 7.6 Hz, $J_{5,6'}$ 7.6 Hz, $J_{5,16}$ 1.2 Hz, H5); 3.60 (s, methoxyl protons); 3.17 (dd, $J_{6,6'}$ 15.3 Hz, $J_{5,6}$ 7.6 Hz, H6); 3.04 (dd, $J_{6,6'}$ 15.3 Hz, $J_{5,6'}$ 7.6 Hz, H6'); 3.03 (dd, $J_{9,9'}$ 18.9 Hz, $J_{8,9'}$ 6.4 Hz, H9'); 2.85 (dq, $J_{11,18}$ 11.0 Hz, $J_{18,19}$ 7.3 Hz, H18); 2.83 (dd, $J_{9,9'}$ 18.9 Hz, $J_{8,9}$ 3.2 Hz, H9); 2.79 (ddq, $J_{8,9'}$ 6.4 Hz, $J_{8,17}$ 6.0 Hz, $J_{8,9}$ 3.2 Hz, H8); 2.21 (dd, $J_{11,18}$ 11.0 Hz, $J_{11,12}$ 9.8 Hz, H11); 2.12 (ddd, $J_{2',3}$ 9.1 Hz, $J_{2,3}$ 3.7 Hz, H3); 2.11 (ddd, $J_{2,3'}$ 7.5 Hz, $J_{2',3'}$ 5.4 Hz, H3;); 1.97 (dddd, $J_{2,2'}$ 14.5 Hz, $J_{2,3'}$ 7.5 Hz, $J_{2,3}$ 3.7 Hz, $J_{1,2}$ 2.8 Hz, H2); 1.58 (d, $J_{5,16}$ 1.2 Hz $(H16)_3$); 1.22 (d, $J_{18,19}$ 7.3 Hz, $(H19)_3$); 1.16 (dddd, $J_{2,2'}$ 14.5 Hz, $J_{1,2'}$ 9.8 Hz, $J_{2',3}$ 9.1 Hz, $J_{2',3'}$ 5.4 Hz, H2'); 1.06 (s, $(H15)_3$); 1.04 (d, $J_{8.17}$ 6.0 Hz, $(H17)_3$); 1.03 (s, $(H14)_3$); 0.17 (ddd, $J_{1,2'}$ 9.8 Hz, $J_{1,12}$ 5.5 Hz, $J_{1,2}$ 2.8 Hz, H1); 0.07 (dd, $J_{1,12}$ 5.5 Hz, $J_{11,12}$ 9.8 Hz, H12). ^{13}C-NMR: see Table 1. MS: essentially similar with that observed for (3). IR: (CCl_4) λ_{max} 1732, 1722, 1715, 1460, 1430, 1400, 1375, 1350, 1310, 1200, 1165, 1120 cm^{-1}. UV: (MeOH) λ_{max} 212 nm (ε 3370). Addition of 5 drops of 10% aq. NaOH gave an additional λ_{max} 285 nm (shoulder) (ε 900).

Base treatment of (2). (I) Methyl ester C (2) (247 mg) was dissolved in pyridine (4 ml) and H_2O (0.5 ml) and heated at 104° under N_2 for 4h. After removal of solvents, the residual oil was separated on preparative radial chromatography eluting with 12% EtOAc-light petroleum to give (a) the methyl ester A (4) (66 mg) as a colourless oil, identical in all respects with a sample

isolated above. Single peak R_t 23.66 min. on capillary GLC (initial temp. 70°, programmed at 30°/min to 130°, then 5°/min. to 275°. ^{1}H-NMR (90 MHz; $CDCl_3$): the following parameters were obtained from NMDR experiments. δ 5.37 (brt, J 7.5 and 1.0 Hz, H5); 3.61 (s, methoxyl protons); 3.45 (ddq, $J_{8,17}$ 6.5 Hz, $J_{8,9'}$ 6.4 Hz, $J_{8,9}$ 3.7 Hz, H8); 3.16 (brs, A part of ABX, H6); 3.07 (brs, B part of ABX, H6'); 3.00 (dq, $J_{11,18}$ 10.6 Hz, $J_{18,19}$ 7.4 Hz, H18); 2.93 (dd, $J_{9,9'}$ 19.8 Hz, $J_{8,9}$ 3.7 Hz, H9); 2.48 (dd, $J_{9,9'}$ 19.8 Hz, $J_{8,9'}$ 6.4 Hz, H9'); 2.19 (dd, $J_{11,18}$ 10.6 Hz, $J_{11,12}$ 8.9 Hz, H11); 2.07 (m, $(H3)_2$); 1.98-1.84 (m, H2); 1.62 (brs, $W_{h/2}$ 3 Hz, $(H16)_3$); 1.21 (d, $J_{18,19}$ 7.4 Hz, $(H19)_3$); 1.05 (s, $(H15)_3$); 1.01 (d, $J_{8,17}$ 6.5 Hz, $(H17)_3$); 0.99 (s, $(H14)_3$); -0.04-0.25 (m, H1 and H12). ^{13}C-NMR: see Table 1. MS: essentially similar with that observed for (2) and (3). IR: (CCl_4) λ_{max} 1730, 1708, 1455, 1430, 1400, 1370 cm^{-1}; and (b) a fraction (101 mg) identical in all respects with a sample of (2).

(II) Methyl ester C (2) (120 mg) was dissolved in d_5-pyridine (1.5 ml) and D_2O (0.75 ml) and heated at 98° under N_2 for 4h. Preparative radial chromatography (12% EtOAc-light petroleum) of the product gave fractions of: (a) the 6,6',8-$^2H_3$ analogue of (4) as a colourless oil, R_t 23.55 min. on capillary GLC (initial temp. 70° programmed at 30°/min. to 130°, then 5°/min. to 275°). ^{1}H-NMR (90 MHz; $CDCl_3$): δ 5.36 (brs, $W_{h/2}$ 5 Hz, H5); 3.61 (s, methoxyl protons); 3.00 (dq, $J_{11,18}$ 11.0 Hz, $J_{18,19}$ 7.4 Hz, H18); 2.92 and 2.48 (dd, $J_{9,9'}$ 20.0 Hz, $(H9)_2$); 2.18 (dd, $J_{11,18}$ 11.0 Hz, $J_{11,12}$ 9.0 Hz, H11); 2.06 (m, $(H3)_3$); 1.98-1.89 (m, H2); 1.62 (d, J 1.2 Hz, $(H16)_3$); 1.21 (d, $J_{18,19}$ 7.4 Hz, $(H19)_3$); 1.04 (s, $(H15)_3$); 1.01 (s, $(H17)_3$); 0.99 (s, $(H14)_3$); -0.04-0.22 (m, H1 and H12). GC-EIMS: R_7 4.9 min. on OV-101 (0.31 mm x 25 m WCOT capillary column, initial temp. 160° programmed at 20°/min.): m/z 351 (M^+, 2%), 292 (1), 264 (3), 200 (11), 172 (33), 152 (28), 151 (11), 140 (100), 137 (24), 123 (20), 109 (30), 96 (20), 95 (27), 82 (32), 81 (29), 70 (32), 69 (42), 67 (22) and (b) the 6-$^2H_1$ analogue of (2) as a colourless oil, R_t 24.30 min. on capillary GLC (initial temp. 70° programmed at 30°/min. to 130°, then 5°/min. to 275°). ^{1}H-NMR (90 MHz; $CDCl_3$): δ 5.32 (brd, J 7.5 Hz, H5); 3.60 (s, methoxyl protons); 3.12 (brd, J 7.5 Hz, H6'); 3.04-2.64 (m, H8, $(H9)_2$, H18); 2.19 (dd, $J_{11,18}$ 11.0 Hz, $J_{11,12}$ 8.9 Hz, H11); 2.08 (m, $(H3)_2$); 1.98-1.80 (m, H2); 1.57 (brs, $W_{h/2}$ 3 Hz, $(H16)_3$); 1.18 (d, $J_{18,19}$ 7.4 Hz, $(H19)_3$); 1.04 (s, $(H15)_3$); 1.01 (d, $J_{8,17}$ 6.5 Hz, $(H17)_3$); 1.01 (s, $(H14)_3$); 0.09-0.27 (m, H1 and H12). GC-EIMS: R_5 5.1 min. on OV-101 (0.31 mm x 25 m WCOT capillary column, initial temp. 160° programmed at 20°/min.): m/z 349 (M^+ 3%), 318 (2), 317 (1), 306 (1), 290 (2), 274 (2), 268 (2), 262 (6), 205 (7), 199 (9), 183 (11), 179 (12), 171 (34), 151 (40), 140 (31), 139 (100), 123 (28), 109 (31), 95 (40), 82 (58), 69 (82), 55 (24).

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