New Diterpenes from *Jatropha divaricata*

Richard W. Denton,[†] Wayne W. Harding,[†] Chadwick I. Anderson,[†] Helen Jacobs,*[,†] Stewart McLean,[‡] and William F. Reynolds*[,‡]

Department of Chemistry, University of the West Indies, Mona, Kingston 7, Jamaica, and Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Canada M5S 3H6

Received February 22, 2001

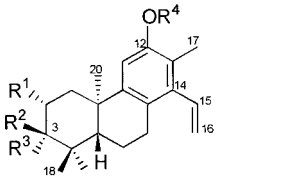
Extracts of the stems of *Jatropha divaricata* have yielded the two new diterpenes *ent*-3*β*,14α-hydroxypimara-7,9(11),15-triene-12-one (**3**) and the rearranged pimarane *ent*-15(13→8)*abeo*-8*β*(ethyl)-pimarane (**4**), which appears to be a new skeletal type. The rare cleistanthane diterpenes spruceanol (**1**) and cleistanthol (**2**) were also obtained.

The large euphorbiaceous genus *Jatropha* L. is recognized as a source of several structural classes of diterpenes,[1] many of which are biologically active, possessing antitumor and cytoxic,[1a] tumor-promoting,[1c] or antimicrobial activity.[1d] Other types of compounds known to occur in *Jatropha* include alkaloids,[2] lignans,[3] and triterpenes.[4]

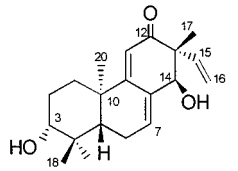
Jatropha divaricata Sw. is a shrub that is endemic to Jamaica.[5] The latex has been screened for protease activity,[6] but prior to this study the plant has not been subjected to thorough phytochemical investigation.

In an initial fractionation of extracts of the aerial parts of *J. divaricata*,[7] the stems and bark were found to contain the diterpenes spruceanol (**1**) and cleistanthol (**2**), *trans*-triacontyl-4-hydroxy-3-methoxy cinnamate, and scopoletin. Compounds characterized from the leaves and twigs were ficaprenol-13, α-tocopherol, plastochromenol-9, and *β*-sitosterol.[7] Spruceanol (**1**)[8] and cleistanthol (**2**)[9] are the only two known naturally occurring members of the cleistanthane series of diterpenes and have each been reported only once previously. Because of the marked bioactivity observed for **1** and **2** isolated from *J. divaricata*, a new supply of plant material was collected. Compounds **1** and **2** were reisolated, and the new diterpenes **3** and **4** were also obtained.

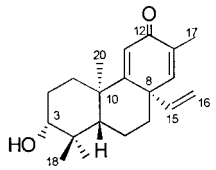
Compound **3**, $C_{20}H_{28}O_3$, was determined to be *ent*-3*β*,14α-hydroxypimara-7,9(11),15-triene-12-one from spectroscopic data. Signals in the ^{1}H NMR spectrum for the C-15,C-16 vinyl group, which is characteristic of pimarane diterpenoids,[10] indicated that **3** was likely to be of this skeletal class. This group provided the starting point for formulation of that portion of the skeleton numbered C-7 through C-14 and the attached methyl groups. This was necessarily based on connectivity data obtained from HSQC and HMBC spectra, as this C-7 to C-14 residue contains no linear protonated sequences apart from the pendant vinyl group. The presence of the protonated sequences C-1 to C-3 and C-5 to C-6 was inferred from associations in the COSY spectrum. These groups were joined to the C-4 center bearing the *gem*-dimethyl group on the basis of HMBC cross-peaks, corroborated by biogenetic arguments, which also enabled linkage of the two major substructures, C-1 to C-6 and C-7 to C-14, to provide structure **3**. The observed long-wavelength UV maximum, 289 nm, provided additional support for the presence of the ketone with extended conjugation. The 2-oxo derivative of **3** has been described as one of the diterpene stress metabolites of cassava roots, *Manihot esculenta* (Euphorbiaceae).[10]



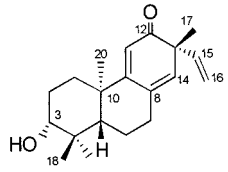
	R^1	R^2	R^3	R^4
1	H	H	OH	H
2	OH	H	OH	H
6	H	=O		CH_3



3



4



5

Compound **4**, $C_{20}H_{28}O_2$, displayed many similarities to **3** in the ^{1}H and ^{13}C NMR spectra. Ring A could be constituted as a $CH_2CH_2CH(OH)$ group flanked by two quaternary carbons, the first bearing an angular methyl group and the other a *gem*-dimethyl group, and these centers are joined by the C-5 methine. A CH_2CH_2 group was attached to this C-5 methine, from COSY data, to provide the substructure C-1 to C-7 and C-10 with the attached methyl groups. Another feature common to **3** and **4** was a vinyl group, and again this was used as the

* To whom correspondence should be addressed. (H.J.) Tel: (876) 935-8409. Fax: (876) 977-1835. E-mail: hjacobs@uwimona.edu.jm. (W.F.R.) Tel/fax: (416) 978-3563. E-mail: wreynold@chem.utoronto.ca.
† University of the West Indies.
‡ University of Toronto.

10.1021/np010098a CCC: $20.00
Published on Web 06/01/2001

reference point for working out the connectivity of ring C in **4**. This vinyl group was attached to a quaternary carbon which showed two- and three-bond correlations to the vinyl protons of two trisubstituted double bonds, one with an α-hydrogen and one with a *β*-hydrogen, both necessarily contained in ring C, which was completed by a carbonyl group. The carbon chemical shift (*δ* 188.7) and IR absorption (1664 cm^{-1}) of this group and the UV maximum of **4** (245 nm) indicated that the carbonyl was probably cross conjugated. The foregoing could be accommodated by two structures. A strong HMBC cross-peak between the signal for the C-20 methyl protons (*δ* 1.14) and that of the quaternary *β*-carbon C-9 (*δ* 172.7) provided decisive evidence for structure **4** and ruled out the alternative with the vinyl, methyl, and carbonyl groups located at positions 9, 12, and 13, respectively. Several additional HMBC cross-peaks were also consistent with structure **4**, e.g., cross-peaks between the carbonyl carbon and both H-14 and the C-17 methyl protons. Compound **4** appears to be a new diterpene skeleton which can formally be named *ent*-15-(13→8)*abeo*-8*β*(ethyl)pimarane.

The relative stereochemistry of **3** and **4** was assigned on the basis of ^{1}H NMR and NOE data. From the coupling constants, H-3 and H-5 are axial in both **3** and **4**. The C-20 methyl group is also axial, as the previously reported[10] *W*-coupling to H-1 axial was observed. Cross-peaks in the T-ROESY spectrum of **3** revealed that this C-20 methyl, H-14, and the vinyl group are on the same face of the molecule. For **4**, a T-ROESY cross-peak between the signal for the C-20 methyl group and that for H-15 established a syn relationship between the vinyl group and the C-20 methyl.

The co-occurrence of the *ent*-pimarane (**3**), cleistanthane (**1**, **2**), and *ent*-15(13→8)*abeo*-8*β*(ethyl)pimarane (**4**) diterpenes in *J. divaricata* implies a close biogenetic link between the three skeletal types; the relationship between the pimaranes and the cleistanthanes is substantiated by the reported conversion, under mild conditions, of a 9-hydroxy-8(14),15-pimaradiene derivative to products with the cleistanthane skeleton.[11] In fact spruceanol (**1**) and compounds **3** and **4** are easily derivable from a common pimarane precursor, *ent*-3*β*-hydroxypimara-8(14),9,15-trien-12-one (**5**): 1,2-shift of the vinyl group enables aromatization of ring C and forms spruceanol (**1**); *β*-epoxidation of the 8,14 double bond and rearrangement of the epoxide produces **3**; thermal [1,3] sigmatropic shift of the vinyl group from C-13 to C-8, suprafacially on the ring, yields compound **4** and accounts for the stereochemistry of the vinyl group.

Spruceanol (**1**) obtained in this study of *J. divaricata* was converted to 12-*O*-methylspruceanone (**6**) by methylation of the phenolic group and oxidation of the C-3 hydroxyl. This compound was strongly levorotatory, $[\alpha]_D$ −68°, and the value of the specific rotation compares favorably with that of the 12-*O*-methylspruceanone prepared from spruceanol isolated from *Cunuria spruceana*;[8,12] the absolute configuration of the dihydro derivative of this compound, from the chiroptical properties, was determined to be 5*S*,10*R*.[8,9] Spruceanol (**1**) from *J. divaricata* therefore has 3*R*,5*S*,10*R* stereochemistry, and on the basis of the close biogenetic relationship between spruceanol and compounds **3** and **4** we propose that the absolute configuration of **3** is 3*R*,5*S*,10*R*,13*R*,14*S* and that of **4** is 3*R*,5*S*,8*S*,10*R*.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. IR spectra were recorded on a Perkin-Elmer 735B spectrophotometer. NMR spectra were obtained on a a Bruker AC200F or Varian UNITY-500 spectrometer with $CDCl_3$ as solvent and TMS as internal standard. COSY, HSQC, and HMBC spectra were obtained in gradient-selected mode using standard Varian software. All mass spectra were obtained on a Micromass VG 70-250S mass spectrometer at 70 eV. Adsorption column chromatography was performed with silica gel 60 (230−400 mesh). TLC analysis utilized Whatman precoated silica gel 60 F_{254} plates. Spots were visualized under UV and by spraying with 4% phosphomolybdic acid in 5% H_2SO_4 followed by heating.

Plant Material. Aerial parts of *Jatropha divaricata* were collected at Ramgoat Cave, Trelawny, in September 1996 and at Mt. Denham, Manchester, Jamaica, in August 1997 and March 2000. Voucher specimens, nos. 34,077−81, 34,675−76, are preserved in the Herbarium, Department of Life Sciences, University of the West Indies, Mona, Jamaica.

Extraction and Isolation. The dried, milled branches (2.9 kg) were extracted by cold percolation with Me_2CO (3 × 6 L) and the extracts evaporated to dryness under reduced pressure to give a gum (30 g). The gum was chromatographed in EtOAc−hexane mixtures of increasing polarity (2%−50%) to produce 12 fractions. Spruceanol (**1**) (1.2 g) and compound **4** (6 mg) were obtained after repeated chromatography of the fraction eluted with 40% EtOAc−hexane. The 50% EtOAc−hexane fraction was rechromatographed in 5% MeOH−$CHCl_3$, and cleistanthol (**2**) (160 mg) was obtained after recrystallization from $CHCl_3$−hexane of one fraction; another fraction yielded compound **3** (13 mg) after further chromatography in 30% Me_2CO−hexane.

***ent*-3*β*,14α-Hydroxypimara-7,9(11),15-triene-12-one (3):** colorless oil: $[\alpha]_D$ +226° (*c* 0.21, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 289 (3.71) nm; IR ν_{max} 3442, 1656, 1640, cm^{-1}; ^{1}H NMR *δ* 6.45 (1H, br m, *W*(1/2) = 12.4 Hz, H-7), 5.93 (1H, dd, *J* = 17.8, 10.8 Hz, H-15), 5.74 (1H, br s, *W*(1/2) = 3.7 Hz, H-11), 5.44 (1H, dd, *J* = 10.8, 0.9 Hz, H-16), 5.29 (1H, dd, *J* = 17.8, 0.9 Hz, H-16), 4.33 (1H, br s, *W*(1/2) = 7.9 Hz, H-14), 3.28 (1H, dd, *J* = 11.5, 4.2 Hz, H-3), 2.48 (1H, dt, *J* = 20.5, 5.6 Hz, H-6*β*), 2.30 (1H, m, H-6α), 1.88 (1H, dt, *J* = 13.7, 3.2, H-1α), 1.81 (1H, m, H-2*β*), 1.79 (1H, m, H-2α), 1.59 (1H, m, H-1*β*), 1.44 (1H, dd, *J* = 13.1, 5.6 Hz, H-5), 1.12 (3H, s, H-17), 1.07 (3H, d, *J* = 0.7 Hz, H-20), 1.04 (3H, s, H-18), 0.96 (3H, s, H-19); ^{13}C NMR *δ* 202.2 (C-12), 164.2 (C-9), 138.9 (C-15), 131.7 (C-7), 131.0 (C-8), 117.9 (C-16), 116.7 (C-11), 78.2 (C-3), 73.1 (C-14), 55.5 (C-13), 47.9 (C-5), 39.3 (C-4), 37.3 (C-10), 34.4 (C-1), 27.4 (C-18), 27.3 (C-2), 24.0 (C-6), 20.7 (C-20), 15.5 (C-19), 14.0 (C-17); HMBC (C/H) 1/2α, 2/1α,3, 3/2α,5,18,19, 4/2α,3,5,18,19, 5/7,6*β*,18,19, 6/5, 7/6*β*, 8/6*β*,11, 9/5,7, 10/5,6*β*,11, 12/15,17, 13/11,15,16-*cis*,16-*trans*,17, 14/15,17, 15/16-*trans*,17, 17/15, 18/5,19, 19/3,5,18, 20/5; EIMS *m*/*z* 316 $[M]^+$ (64), 301 (21), 299 (37), 283 (48), 211 (43), 185 (49), 176 (63), 157 (70), 145 (62), 131 (66), 121 (58), 105 (78), 95 (83), 77 (57), 69 (67), 55 (100); HREIMS *m*/*z* 316.2030 (calcd for $C_{20}H_{28}O_3$, 316.2039).

Compound 4: colorless oil: $[\alpha]_D$ +133° (*c* 0.27, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 245 (3.82) nm; IR ν_{max} 3432, 1664, 1628, cm^{-1}; ^{1}H NMR *δ* 6.15 (1H, q, *J* = 1.4 Hz, H-14), 6.14 (1H, br s, H-11), 5.44 (1H, ddd, *J* = 17.5, 10.3, 0.9, H-15), 5.20 (1H, d, *J* = 17.5 Hz, H-16), 5.19 (1H, d, *J* = 10.3 Hz, H-16), 3.22 (1H, dd, *J* = 11.6, 4.3 Hz, H-3), 2.37 (1H, ddd, *J* = 13.7, 3.3, 3.3 Hz, H-7α), 1.86 (3H, d, *J* = 1.4 Hz, H-17), 1.81 (1H, m, H-2*β*), 1.80 (2H, m, H-1α, H-6α), 1.74 (1H, m, H-6*β*), 1.72 (1H, m, H-2α), 1.58 (1H, m, H-1*β*), 1.42 (1H, ddd, *J* = 13.7, 10.6, 3.3 Hz, H-7*β*), 1.14 (3H, brs, *W*(1/2) = 2.4 Hz, H-20), 1.01 (3H, s, H-18), 0.97 (1H, dd, *J* = 12.0, 2.5 Hz, H-5), 0.86 (3H, s, H-19); ^{13}C NMR *δ* 188.7 (C-12), 172.7 (C-9), 150.9 (C-14), 140.0 (C-15), 131.4 (C-13), 122.0 (C-11), 113.7 (C-16), 78.3 (C-3), 53.1 (C-5), 47.0 (C-8), 41.4 (C-10), 39.6 (C-4), 36.0 (C-1), 33.7 (C-7), 28.3 (C-18), 27.4 (C-2), 21.4 (C-20), 17.9 (C-6), 15.6 (C-19), 15.2 (C-17); HMBC (C/H) 1/20, 3/1,2α,2*β*,5,18,19, 4/5,18,19, 5/6α,6*β*,7α,18,19,20, 7/14,15, 8/6*β*,7α,11,14,15,16-*trans*, 9/7α,11,14,20, 10/2α,6α,11, 11/7*β*, 12/11,14,17, 13/11,14,17, 14/17, 15/7*β*,16-*trans*, 17/14, 18/19, 19/5,18; EIMS *m*/*z* 300 $[M]^+$ (100), 285 (48), 267 (26), 257 (29), 239 (23), 213

(20), 199 (20), 185 (24), 171 (26), 161 (41), 147 (56), 133 (44), 118 (41), 97 (28), 91 (36), 83 (35), 69 (50), 55 (67); HREIMS *m*/*z* 300.2084 (calcd for $C_{20}H_{28}O_2$, 300.2089).

Acknowledgment. We thank Mr. Patrick Lewis and Dr. Vonna Caleb-Drayton for assistance with plant collection and identification. Financial support from the UWI Board for Postgraduate Studies, The Netherlands Government, and the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

Supporting Information Available: Physical and spectroscopic data for spruceanol (**1**), cleistanthol (**2**), and 12-*O*-methylspruceanone (**6**) and procedure for the preparation of **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Evans, F. J.; Taylor, S. E. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: Wein, 1983; Vol. 44, pp 1−99. (b) Brum, R. L.; Honda, N. K.; Mazarin, S. M.; Hess, S. C.; Cavalheiro, A. J.; Delle Monache, F. *Phytochemistry* **1998**, *48*, 1225−1227 (c) Adolf, W.; Opferkuch, H. J.; Hecker, E. *Phytochemistry* **1984**, *23*, 129−132. (d) Naenchomnong, B. W.; Thebtaranonth, Y.; Wiriyachitra, P.; Okamato, K. T.; Clardy, J. *Tetrahedron Lett*. **1986**, *27*, 2439−2442.

(2) Staubmann, R.; Schubert-Zsilavecz, M.; Hiermann, A.; Kartnig, T. *Phytochemistry* **1999**, *50*, 337−339.

(3) (a) Schmeda-Hirschmann, G.; Tsichritzis, F.; Jakupovic, J. *Phytochemistry* **1992**, *31*, 1731−1735. (b) Das, B.; Rao, S. P.; Srinivas, K. V. N. S.; Das, R. *Phytochemistry* **1996**, *41*, 985−987.

(4) Tinto, W. F.; John, L. M. D.; Reynolds, W. F.; McLean, S. *J. Nat. Prod*. **1992**, *55*, 807−809.

(5) Adams, C. D. *Flowering Plants of Jamaica*; The University Press: Glasgow, 1972; p 403.

(6) Reddy, S. M.; Provindra, C. M. *Natl. Acad. Sci. Lett*. **1983**, *6*, 183−184; *Chem. Abstr*. **1983**, *100*, 153850k.

(7) Denton, R. W. Phytochemistry of Endemic Species from Jamaican Euphorbiaceae and Asclepiadaceae. M. Philos. Thesis, University of the West Indies, Mona, Jamaica, 1999; pp 35−127.

(8) Gunasekera, S. P.; Cordell, G. A.; Farnsworth, N. R. *J. Nat. Prod*. **1979**, *42*, 658−662.

(9) McGarry, E. J.; Pegel, K. H.; Phillips, L.; Waight, E. S. *J. Chem. Soc. (C)* **1971**, 904−909.

(10) Sakai, T.; Nakagawa, Y. *Phytochemistry* **1988**, *27*, 3769−3779.

(11) Ellestad, G. A.; Kunstmann, M. P.; Morton, G. O. *J. Chem. Soc., Chem. Commun*. **1973**, 312−313.

(12) Compound **1** from *J. divaricata* appears to be identical to spruceanol reported from *Cunuria spruceana*,[8] although our specific rotation and the *J* values for H-3 differ from those given in ref 8.

NP010098A