Phytochemistry, Vol. 27, No. 1, pp. 233–235, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00
Pergamon Journals Ltd.

3β-(3,4-DIHYDROXYCINNAMOYL)-ERYTHRODIOL AND 3β-(4-HYDROXYCINNAMOYL)-ERYTHRODIOL FROM *LARREA TRIDENTATA*

HUI-ZHENG XUE, ZHI-ZHEN LU, CHOHACHI KONNO, D. DOEL SOEJARTO, GEOFFREY A. CORDELL, HARRY H. S. FONG and WENDY HODGSON*

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, U.S.A; *Desert Botanical Garden, 1201 North Galvin Parkway, Phoenix, AZ 85008, U.S.A.

(*Received* 10 *March* 1987)

Key Word Index—*Larrea tridentata*; Zygophyllaceae; Creosote bush; structure elucidation; triterpene esters; 3β-(3,4-dihydroxycinnamoyl)-erythrodiol; 3β-(4-hydroxycinnamoyl)-erythrodiol.

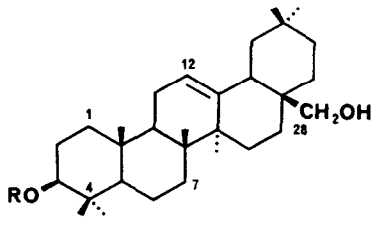
Abstract—From the stems of *Larrea tridentata* two new triterpenoids have been obtained, whose stereostructures have been elucidated on the basis of chemical and physical evidence.

INTRODUCTION

As part of our continuing studies on the isolation and structure elucidation of fertility regulating compounds from plants, we have investigated *Larrea tridentata* (DC) Coville* which has been used as a contraceptive agent [3], and also displays uterine relaxation activity *in vitro* [4]. Previous chemical studies have established that this species contains flavonoids, lignans, volatile oils and saponins [5–13]. Here we report the isolation and structure elucidation of two new triterpenes, **1** and **2**.

RESULTS AND DISCUSSION

Dried and ground stems of *L. tridentata* were extracted with methanol and the extract repeatedly chromatographed on silica gel to afford three terpenes, one of which was readily identified as β-sitosterol. The second compound (triterpenoid A), mp 270–271°, had a molecular formula $C_{39}H_{56}O_5$ (M^+, m/z 604.4049; calcd 604.4128 for $C_{39}H_{56}O_5$). Its IR spectrum showed the presence of hydroxyl groups (ν_{max} 3340 cm^{-1}) and an α,β-unsaturated ester moiety (1675 and 1259 cm^{-1}). In the ^1H NMR spectrum in $(CD_3)_2CO$, AB type signals (δ6.31 and 7.55, J = 16 Hz) due to protons on a *trans*-disubstituted double bond and ABC type signals (δ6.88, J = 8.4 Hz; 7.02, J = 8.4 and 1.9 Hz; and 7.18 J = 1.9 Hz) from three aromatic protons were noted. From these data, the presence of a 3,4-dihydroxycinnamoyl moiety in triterpenoid A was suggested. Support for this came from the observation of UV maxima at 216, 244, 255, 305 and 372 nm, a very strong ion at m/z 163, and a series of ^{13}C NMR signals similar to those of caffeic acid (Table 1).



R

1 3,4 – Dihydroxycinnamoyl

2 4 – Hydroxycinnamoyl

3 H

The ^1H NMR spectrum showed seven tertiary methyl signals (δ0.90, 0.90, 0.92, 0.97, 1.00, 1.03 and 1.23), an AB system (δ3.14 and 3.56, J = 10.7 Hz) due to a hydroxymethyl group, a doublet of doublets (δ4.58 J = 11.3 and 4.9 Hz) due to a proton on a carbon bearing an ester oxygen, and a triplet (δ5.20, J = 3.3 Hz) due to an olefinic proton. These signals were similar to those of erythrodiol and its derivatives [14].

Comparison of the ^{13}C NMR signals of triterpenoid A, erythrodiol, erythrodiol diacetate and caffeic acid (Table 1) [14–17] showed that triterpenoid A is 3β-(3,4-dihydroxycinnamoyl)-erythrodiol (**1**). Alkaline hydrolysis afforded a neutral compound identified as erythrodiol (**3**).

The third compound (triterpenoid B), mp 287–289°, displayed a M^+ at m/z 588 in agreement with a molecular formula of $C_{39}H_{56}O_4$. In the IR spectrum absorbances for hydroxyl groups (ν_{max} 3395 cm^{-1}) and an α,β-unsaturated ester group (ν_{max} 1676 and 1264 cm^{-1}) were observed as in **1**. In the ^1H NMR spectrum, AB type signals (δ6.38 and 7.61, J = 16.8 Hz) due to protons on a *trans*-disubstituted double bond were observed together with A_2B_2 type signals at δ6.90 and 7.51 (J = 8.9 Hz) due to protons on a *p*-substituted aromatic ring. These data suggested that triterpenoid B contained a 4-hydroxy cin-

*In the more recent literature there is a tendency towards the recognition of *L. tridentata* Cov., native to southwestern U.S. and northern Mexico, as a separate species from *L. divaricata* Cav., native to northwestern Argentina [1, 2]. In this article we have used *L. tridentata* for the North American species.

Table 1. ^{13}C NMR data for compound **1** and related compounds

	1 (pyridine-d_5)	Erythrodiol (**3**) ($CDCl_3$)	Erythrodiol diacetate ($CDCl_3$)	3,4-Dihydroxy-cinnamic acid (pyridine-d_5)
1	38.2	38.6	38.2	
2	23.4	27.2	23.4	
3	80.5	79.0	80.7	
4	37.7	38.8	37.6	
5	55.6	55.2	55.1	
6	18.6	18.4	18.1	
7	32.8	32.6	32.4	
8	40.1	39.8	39.6	
9	47.9	47.6	47.4	
10	37.1	36.9	36.7	
11	24.2	23.6	23.4	
12	122.4	122.3	122.5	
13	145.2	144.2	143.3	
14	42.1	41.7	41.5	
15	26.1	25.6	25.5	
16	22.9	22.0	22.1	
17	34.8	36.9	35.7	
18	42.8	42.3	42.4	
19	47.1	46.5	46.1	
20	31.3	31.0	30.7	
21	34.8	34.1	33.9	
22	31.9	31.0	31.3	
23	28.3	28.1	27.9	
24	17.0	15.5	16.6	
25	15.7	15.5	15.5	
26	17.3	16.7	16.6	
27	26.3	25.9	25.8	
28	68.8	69.7	70.5	
29	33.6	33.2	33.0	
30	23.9	23.6	23.4	
1′	127.0			127.4
2′	115.6			115.9
3′	147.8			147.9
4′	150.5			150.3
5′	116.8			117.0
6′	122.1			122.0
7′	145.8			145.4
8′	115.9			116.9
9′	167.4			169.9

namoyl moiety and this was further confirmed by a fragment ion at m/z 147 in the mass spectrum which was otherwise essentially identical with that of **1**.

The ^1H NMR spectrum of triterpenoid B also showed seven methyl singlets (δ0.90, 0.90, 0.92, 0.97, 1.00, 1.03 and 1.23), an AB system at δ3.14 and 3.51 (J = 10.8 Hz) due to a hydroxymethyl group, an ester methine doublet of doublets (δ4.59, J = 11.7 and 4.7 Hz), and a triplet (δ5.20, J = 3.4 Hz) for a vicinal olefinic proton. These signals were identical with those of **1**. Triterpenoid B is therefore 3β-(4-hydroxycinnamoyl)-erythrodiol (**2**).

EXPERIMENTAL

General methods. Mps: uncorr. ^1H NMR and ^{13}C NMR: 360 MHz and 200 MHz respectively, TMS as int. standard.

Plant material. The stems of *L. tridentata* were collected by one of us (W.H.) in the vicinity of Phoenix, AZ in June 1983. They were identified by W.H. and D.D.S. and herbarium specimens are deposited in the herbarium of the Desert Botanical Garden, Phoenix, AZ, U.S.A. and the John G. Searle Herbarium, Field Museum of Natural History, Chicago, IL, U.S.A.

Isolation of triterpenoids. The dried, ground stems of *L. tridentata* (34.2 kg) were exhaustively extracted with MeOH at room temp. and the combined extracts evapd to afford a MeOH extract (3.49 kg). A sample of the extract (3.33 kg) was chromatographed over silica gel (5 kg) eluting with $CHCl_3$ and mixtures of $CHCl_3$–MeOH of increasing polarity to afford $CHCl_3$ (158 g), $CHCl_3$–MeOH (19:1, 88 g), $CHCl_3$–MeOH (9:1, 174 g), $CHCl_3$–MeOH (4:1, 105 g) and MeOH (2430 g) fractions. The $CHCl_3$–MeOH (19:1) fraction was repeatedly chromatographed over silica gel. Elution with C_6H_6–EtOAc (9:1) and recrystallization from Me_2CO afforded β-sitosterol (60 mg), identified by direct comparison with an authentic sample.

Successive elution with C_6H_6–EtOAc (9:1) and recrystallization from Me_2CO afforded triterpenoid B (**2**, 2.5 mg, 8×10^{-6}%) as colourless needles, mp 287–289°; $[\alpha]_D^{25}$ +62 (MeOH; c 0.13); IR ν_{max}^{KBr} cm^{-1}: 3395, 1676, 1264; UV λ_{max}^{MeOH} nm: 225 (logε 4.26), 312 (4.30); (MeOH + NaOH) 228 (4.43), 311 (4.13), 359 nm (4.52). ^1H NMR, (360 MHz, $(CD_3)_2CO$): δ0.90 (6H, *s*), 0.92 (3H, *s*), 0.97 (3H, *s*), 1.00 (3H, *s*), 1.03 (3H, *s*), 1.23 (3H, *s*), 3.14 (1H, *m*, H-28), 3.51 (1H, *m*, H-28), 4.59 (1H, *dd*, J = 11.7, 4.7 Hz, H-3α), 5.20 (1H, *t*, J = 3.4 Hz, H-12), 6.38 (1H, *d*, J = 16.8 Hz, H-8′), 6.90 (2H, *d*, J = 8.9 Hz, H_2-3′,5′), 7.51 (2H, *d*, J = 8.9 Hz, H_2-2′,6′), and 7.61 (1H, *d*, J = 16.8 Hz, H-7′); EIMS 70 eV, m/z (rel. int.): 588 $[M]^+$ (1), 570 (2), 424 (3), 409 (1), 393 (3), 381 (1), 286 (1), 234 (18), 216 (10), 203 (100), 191 (25), 187 (5), 175 (5), 147 (50), 135 (15), 133 (7), 119 (12), 107 (7), 105 (7), 95 (8), 93 (5), 91 (6), 81 (9), 69 (14), 55 (9), 43 (7) and 41 (7).

The $CHCl_3$–MeOH (9:1) fraction was rechromatographed over silica gel eluting with $CHCl_3$–MeOH (93:7) and the resulting main fraction was further chromatographed over silica gel. Elution with C_6H_6–EtOAc (7:3) and recrystallization from Me_2CO afforded triterpenoid A (**1**, 250 mg, 8×10^{-4}%) as colourless needles, mp 270–271°; $[\alpha]_D^{25}$ +70° (MeOH; c 0.55); IR ν_{max}^{KBr} cm^{-1}: 3340, 1675, 1259; UV λ_{max}^{MeOH} nm: 216 (logε 4.22), 244 (4.04), 301 (4.15), 328 (4.25); (MeOH + NaOH) 224 (4.57), 255 (4.31), 305 (4.02), 372 nm (4.36). ^1H NMR [360 MHz, $(CD_3)_2CO$]: δ0.90 (6H, *s*), 0.92 (3H, *s*), 0.97 (3H, *s*), 1.00 (3H, *s*), 1.03 (3H, *s*), 1.23 (3H, *s*), 3.14 (1H, *d*, J = 10.7 Hz, H-28), 3.56 (1H, *d*, J = 10.7 Hz, H-28), 4.58 (1H, *dd*, J = 11.3 and 4.9 Hz, H-3α), 5.20 (1H, *t*, J = 3.3 Hz, H-12), 6.31 (1H, *d*, J = 16.0 Hz, H-8′), 6.88 (1H, *d*, J = 8.4 Hz, H-5′), 7.02 (1H, *dd*, J = 8.4 and 1.9 Hz, H-6′), 7.18 (1H, *d*, J = 1.9 Hz, H-2′), and 7.55 (1H, *d*, J = 16.0 Hz, H-7′); ^{13}C NMR, see Table 1; EIMS 70 eV, m/z (rel. int.): 604 $[M]^+$ (0.7), 586 (1), 424 (3), 409 (2), 393 (4), 381 (1), 286 (2), 234 (28), 216 (21), 203 (100), 191 (47), 187 (12), 175 (17), 163 (100), 147 (16), 135 (18), 133 (17), 119 (22), 107 (22), 105 (23), 95 (27), 93 (20), 91 (12), 81 (28), 69 (43), 55 (29), 43 (24), and 41 (24).

Hydrolysis of triterpenoid A. To triterpenoid A (**1**, 30 mg) was added 5% K_2CO_3 in MeOH–H_2O (5 ml; 1:1) and the mixt. left at room temp. overnight. Work-up in the usual way afforded a product which was chromatographed over silica gel to afford erythrodiol (**3**, 8 mg), which was identified by comparison (TLC, mmp, IR, ^1H NMR, ^{13}C NMR and MS) with an authentic sample.

Acknowledgements—This work was supported, in part, by a grant (HRP project 77918C) from the Task Force on Indigenous Plants for Fertility Regulation, Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization, Geneva, Switzerland. The authors also thank the Research Resources Center of the University of Illinois at Chicago for the provision of NMR and mass spectro-

scopic facilities and Dr J. Liesch of Merck Sharp and Dohme Research Laboratories, Rahway, NJ for the provision of high resolution mass spectral data.

REFERENCES

1. Solbrig, O. (1977) In *The Creosote Bush; The Biology and Chemistry of Larrea in the New World Deserts* (Mabry, T. J., Hunziker, J. H. and Di Feo, Jr., D. R., eds) p. 1. Dowden, Hutchinson and Ross, Stroudsburg, PA.
2. Hunziker, J. H., Palacios, R. A., Poggio, L., Naranjo, C. A. and Wang, T. W. (1977) In *The Creosote Bush; The Biology and Chemistry of Larrea in the New World Deserts* (Mabry, T. J., Hunziker, J. H. and Di Feo, Jr., D. R., eds) p. 10. Dowden Hutchinson and Ross, Stroudsburg, PA.
3. Moser, M. B. (1970) *The Kiva* **35**, 201.
4. Moreno, M. S. F. (1972) *C.R. Seances Soc. Biol. Ses. Fil.* **87**, 563.
5. Waller, C. W. and Gisvold, O. (1945) *J. Am. Pharm. Assoc., Sci. Ed.* **34**, 78.
6. Page, J. O. (1955) *Anal. Chem.* **27**, 1266.
7. Smart, C. R., Hogle, H. H., Robins, R. K., Broom, A. D. and Bartholomew, D. (1969) *Cancer Chemother. Rep.*, 1 **53**, 147.
8. Chirikdjian, J. J. (1973) *Z. Naturforsch.* **28C**, 32.
9. Chirikdjian, J. J. (1973) *Sci. Pharm.* **41**, 206.
10. Gisvold, O. and Thaker, E. (1974) *J. Pharm. Sci.* **63**, 1905.
11. Seigler, D. S., Jakupcak, J., and Mabry, T. J. (1974) Phytochemistry **13**, 983.
12. Sakakibara, M., DiFeo, D. R., Nakatani, N., Timmermann, B. and Mabry, T. J. (1976) *Phytochemistry* **15**, 727.
13. Mabry, T. J., DiFeo, D. R., Sakakibara, M., Bohnstedt, C. F. and D. S. Seigler, In *The Creosote Bush: The Biology and Chemistry of Larrea in the New World Deserts* (Mabry, T. J., Hunziker, J. H. and DiFeo, D. R., eds) p. 115. Dowden, Hutchinson and Ross, Stroudsburg, PA.
14. Gonzalez, A. G., Fraga, B. M., Gonzalez, P., Hernandez, M. G. and Ravelo, A. G. (1981) *Phytochemistry* **20**, 1919.
15. Benzs, I., Beizaee, N., Vanek, T., Vana, J. and Herout, V. (1981) *Phytochemistry* **20**, 2438.
16. Massow, F. and Smith, M. A. R. (1976) *J. Chem. Soc., Perkin Trans. II* 997.
17. Harborne, J. B. and Mabry, T. J. (1982) *The Flavonoids: Advances in Research*. Chapman & Hall, London.