Phytochemistry, Vol. 22, No. 11, pp. 2617–2619, 1983.
Printed in Great Britain.

0031–9422/83 $3.00+0.00

2α-*ISO*-VALEROYLOXYEPERUIC ACID, A DITERPENE FROM *EUPATORIUM PETIOLARE**

J. S. CALDERÓN, L. QUIJANO, M. GARDUÑO, F. GÓMEZ and T. RIOS

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán, 04510 México, D.F.

(*Received* 18 *March* 1983)

Key Word Index—*Eupatorium petiolare*; Compositae; Eupatorieae; labdane type diterpenes; 2α-*iso*-valeroyloxyeperuic acid.

Abstract—The diterpene acid 2α-*iso*-valeroyloxyeperuic acid was isolated from the aerial parts of *Eupatorium petiolare*. Its structure and stereochemistry were elucidated by spectroscopic methods and chemical correlation with eperuic acid.

INTRODUCTION

As a continuation of our chemical investigations of the genus *Eupatorium* (tribe Eupatorieae) [1–4], we have undertaken the study of *Eupatorium petiolare*, and have isolated a new labdane type diterpene which was shown to be 2α-*iso*-valeroyloxyeperuic acid (**1a**), together with the known kaurenoic acid, 6-methoxy salicylic benzyl ester and a mixture of taraxasteryl palmitate, stearate and arachidate.

RESULTS AND DISCUSSION

2α-*iso*-Valeroyloxyeperuic acid (**1a**) $C_{25}H_{42}O_4$ was isolated as a colourless viscous liquid which was shown to be a terpene acid by the presence of absorptions at 3500–2400 and 1700 cm^{-1}; this was confirmed by formation of the methyl ester (**1b**) with diazomethane. The presence of two broad singlets at δ 4:50 and 4.84 in the ^1H NMR spectrum, together with an absorption band at 890 cm^{-1} in the IR spectrum, indicated the presence of an exocyclic methylene group.

The ^1H NMR spectrum also showed four methyl groups, a secondary one at δ 0.91 (*d*, $J = 6$ Hz) and three tertiary ones at δ 0.87, 0.95 and 1.00, which together with the exocyclic methylene indicated a labdane skeleton. The presence of an *iso*-valerate side chain ester (1730 cm^{-1}) was indicated by the mass spectral ion peak at *m/z* 304 $[M-C_5H_{10}O_2]^+$ (12.4%) and the signal for the *iso*-propyl group at δ 0.94 in the ^1H NMR spectrum of **1a**. A quintet at δ 5.18, $J = 5$ Hz, was assigned to H-2 bearing the *iso*-valerate group, since this signal was shifted upfield to δ 4.16 upon hydrolysis. The multiplicity and coupling constants of this signal can only arise by interaction of an equatorial proton with two neighboring axial and two equatorial protons. Hence the *iso*-valerate group must be placed at C-2 and α-oriented. Concerning the stereochemistry at C-9 and C-10 the CD spectrum of **1h** showed at λ_{max} 283 nm a positive ($[\theta] = +8854$) Cotton effect similar to that exhibited by **1i** obtained from eperuic acid and opposite to that exhibited by **2** obtained from labdanolic acid [5], hence the stereochemistry at C-9 and C-10 must be α as in eperuic acid.

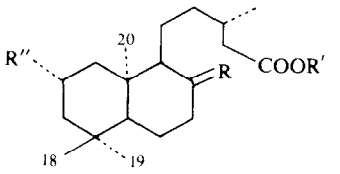
Confirmation of the structure and stereochemistry of **1a** was achieved by chemical correlation with eperuic acid, a diterpene isolated from *Eperua falcata* [6], whose stereochemistry [7] and absolute configuration at C-13 [8] are known. Alkaline hydrolysis of **1a** afforded *iso*-valeric acid and **1c**, which was treated with diazomethane to give the methyl ester (**1d**). Jones oxidation of **1d** furnished the keto-ester (**1f**) which was reduced under Wolff–Kishner conditions and the reaction product esterified with diazomethane to afford methyl eperuate (**1g**) whose ^1H NMR spectrum and specific rotation were identical to those previously published [6, 9].

EXPERIMENTAL

Mps are uncorr. Known compounds were identified by comparison of the IR and ^1H NMR spectra. Analysis was determined by Dr. F. Pascher, Germany.

Eupatorium petiolare Moc, was collected in Mexico City at UNAM in March 1977. A voucher Calderon-2 has been deposited at the Herbarium of the Instituto de Biologia (UNAM), Mexico. The air-dried plant material, leaves and flowers (940 g) were extracted with petrol (twice), and then with $CHCl_3$. The petrol extract, after removing long chain hydrocarbons (177.6 g) was separated by CC over 2.5 kg silica gel, using petrol–C_6H_6 and C_6H_6–EtOAc mixtures as eluants. Fractions eluted with petrol–C_6H_6 (75:25) gave a mixture of taraxasteryl esters (20 g). A sample of 1 g was hydrolysed with NaOH in EtOH, giving taraxasterol and a mixture of palmitic, stearic and arachidic acids which were identified by its MS which exhibited molecular ions at *m/z* 256 ($C_{16}H_{32}O_2$), 285 ($C_{18}H_{36}O_2$) and 312 ($C_{20}H_{40}O_2$).

*Contribution No. 634 from Instituto de Quimica, U.N.A.M. (México).



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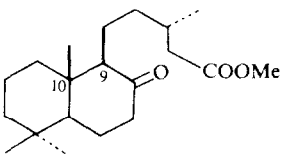
1a R = CH_2, R' = H, R'' = O–*isc*–Valeroyl
1b R = CH_2, R' = Me, R'' = O–*iso*–Valeroyl
1c R = CH_2, R' = H, R'' = OH
1d R = CH_2, R' = Me, R'' = OH
1e R = CH_2, R' = Me, R'' = OAc
1f R = CH_2, R' = Me, R'' = O
1g R = CH_2, R' = Me, R'' = H
1h R = O, R' = Me, R'' = OAc
1i R = O, R' = Me, R'' = H



2

Further elution with C_6H_6 afforded 4.91 g of 6-methoxy salicylic benzylester as a crystalline solid mp 38–39° (lit. 40° [10]) and 5.6 g of kaurenoic acid.

2α-iso-valeroyloxy eperuic acid (**1a**). Chromatography fractions eluted with C_6H_6–EtOAc (98:2) were combined to give 25 g of crude **1a**. A 10 g sample of this material was rechromatographed on 250 g of silica gel to give 6.04 g of **1a** as a colourless oil. IR ν_{max}^{film} cm^{-1}: 3500–2400, 1730, 1700, 1180, 890. ^1H NMR (100 MHz, $CDCl_3$): δ 0.87 (3H, *s*, H-20), 0.91 (3H, *d*, J = 6 Hz, H-16), 0.95 (3H, *s*, H-19), 1.00 (3H, *s*, H-18), 0.94 (6H, *d*, J = 6 Hz, *iso*-propyl), 4.50 (1 H, *br s*, H-17), 4.84 (1 H, *br s*, H-17'), 5-18 (1 H, *m*, H-2). EIMS 70 eV *m/z* (rel. int.): no $[M]^+$, 304 $[M - C_5H_{10}O_2]^+$ (12.4), 135 $[C_8H_{15}]^+$ (100), 122 (16).

$$[\alpha]_\lambda^{25} = \frac{-26.5 \quad -27.0 \quad -30.5 \quad -50.0 \quad -73.0}{589 \quad 578 \quad 546 \quad 436 \quad 365} \quad (CHCl_3;\ c,\ 0.20).$$

Methyl ester (**1b**). Compound **1a** (100 mg) was treated with an ethereal soln of CH_2N_2 to yield 90 mg **1b** as an oil. $[\alpha]_D^{25}$ −28.1° ($CHCl_3$; *c* 0.32). IR ν_{max}^{film} cm^{-1}: 1735, 1645, 890. ^1H NMR (80 MHz, $CDCl_3$): δ, overlapped signals at 0.9 (18H, *m*, H-16, H-18, H-19, H-20, *iso*-propyl), 3.65 (3H, *s*, OMe), 4.50 (1H, *br s*, H-17) 4.85 (1H, *br s*, H-17'), 5.17 (1H, *quintet*, J = 5 Hz, H-2). EIMS 70 eV *m/z* (rel. int.): no $[M]^+$, 318 $[M - C_5H_{10}O_2]^+$ (19), 189 (20.6), 175 (17.8), 135 $[C_8H_{15}]^+$ (100).

2α-Hydroxy eperuic acid (**1c**). To a soln of 3.037 g **1a** in 50 ml of MeOH, 1.6 g NaOH were added. The reaction was monitored by TLC. After 4 hr the reaction was worked up as usual to give 1.572 g of **1c** as a crystalline compound, mp 127–128°. $[\alpha]_D^{25}$ −53.3° ($CHCl_3$; *c* 0.30). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–2400, 1705, 1640, 890. ^1H NMR (100 MHz, $CDCl_3$): δ 0.92 (6H, *s*, H-20, H-19), 0.97 (3H, *d*, J = 6 Hz, H-16), 0.98 (3H, *s*, H-18), 4.16 (1H, *quintet*, J = 5 Hz, H-2), 4.50 (1H, *br s*, H-17), 4.82 (1H, *br s*, H-17'), 6.23 (1H, *br*, –COOH). EIMS (probe) 70 eV *m/z* (rel. int.): 322 $[M]^+$ (0.3), 304 $[M - H_2O]^+$ (7.5), 289 $[M - H_2O - Me]^+$ (6.3), 135 $[C_8H_{15}]^+$ (100), 107 (48.4), 93 (49.5). (Found: C, 74.14; H, 10.60; O, 15.10. $C_{20}H_{34}O_3$ requires: C, 74.49; H, 10.63; O, 14.89%.) The mother liquors of the later hydrolysis were methylated with CH_2N_2 to give 200 mg methyl *iso*-valerate.

2α-Hydroxy-methyl eperuate (**1d**). Esterification of 1.017 g **1c** with CH_2N_2 afforded 0.99 g of **1d** as an oil. $[\alpha]_D^{25}$ = −48.9° ($CHCl_3$; *c* 0.45). IR ν_{max}^{film} cm^{-1}: 3450, 1730, 1640, 890. ^1H NMR (100 MHz, $CDCl_3$): δ 0.92 (6H, *s*, H-20, H-19), 0.93 (3H, *d*, J = 6 Hz, H-16), 0.98 (3H, *s*, H-18), 3.64 (3H, *s*, OMe), 4.14 (1H, *quintet*, J = 5 Hz, H-2), 4.48 (1H, *br s*, H-17), 4.82 (1H, *br s*, H-17'). EIMS 70 eV *m/z* (rel. int.): 336 $[M]^+$ (1.5), 318 $[M - H_2O]^+$ (22), 303 $[M - H_2O - Me]^+$ (17), 193 (24), 175 (31), 135 $[C_8H_{15}]^+$ (100), 109 (63).

2α-Acetyloxy-methyl eperuate (**1e**). A 650 mg sample of **1d** acetylated with Ac_2O–C_5H_5N as usual gave the oily acetate **1e** (700 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1640, 890. ^1H NMR (100 MHz, $CDCl_3$): δ 0.87 (1H, *s*, H-20), 0.91 (1H, *s*, H-19), 0.97 (*d*, J = 6 Hz, H-16), 0.98 (3 H, *s*, H-18), 2.1 (3 H, *s*, Ac) 3.66 (3H, *s*, OMe), 4.49 (1H, *br s*, H-17), 4.87 (1H, *br s*, H-17'), 5.2 (1H, *quintet*, J = 5 Hz, H-2). EIMS 70 eV *m/z* (rel. int.): no $[M]^+$, 318 $[M - AcOH]^+$ (1.3), 303 $[M - AcOH - Me]^+$, 135 $[C_8H_{15}]^+$ (43), 43 $[MeCO]^+$ (100).

Ozonolysis of **1e**. A soln of 700 mg **1e** in $CHCl_3$ was ozonized for 10 min. The ozonide was decomposed with triphenylphosphine, the solvent removed and the residue purified by prep. TLC giving 300 mg **1h**. IR ν_{max}^{film} cm^{-1}: 1730, 1250. ^1H NMR (100 MHz, $CDCl_3$): δ 0.91 (611, *s*, H-19, H-20), 0.98 (3H, *d*, J = 6 Hz, H-16), 1.04 (3H, *s*, H-18), 2.01 (3H, *s*, Ac), 3.65 (3H, *s*, OMe) 5.16 (1H, *quintet*, J = 4 Hz, H-2). EIMS 70 eV *m/z* (rel. int.): 380 $[M]^+$ (2.5), 252 (37.5), 192 (25), 177 (100), 135 (C_8H_{15}) (47), 43 $[MeCO]^+$ (64). CD (MeOH) $[\theta]_{285}$ + 8854.

2-Oxo-methyl eperuate (**1f**). To a soln of **1d** (150 mg) in Me_2CO (10 ml) Jones reagent was added dropwise with cooling by ice, the reaction being monitored by TLC. After the usual work-up, the residue was purified by TLC to give 100 mg **1f**, oil, IR ν_{max}^{film} cm^{-1}: 1740, 1710, 1640, 890. ^1H NMR (100 MHz, $CDCl_3$): δ 0.70 (3H, *s*, H-20), 0.85 (3H, *s*, H-19), 0.93 (3H, *d*, J = 6 Hz, H-16), 1.06 (3H, *s*, H-18), 3.64 (3H, *s*, OMe), 4.54 (1H, *br s*, H-17), 4.88 (1H, *br s*, H-17'). EIMS 70 eV *m/z* (rel. int.): 334 $[M]^+$ (5.7), 319 $[M - Me]^+$ (20.5), 236 (32), 203 (50), 151 $[C_{10}H_{15}O]^+$ (100).

Methyl eperuate (**1g**). A 100 mg sample of **1f** was heated for 3 hr with ethylene glycol (3 ml), KOH (350 mg) and hydrazine hydrate (250 mg) in a tube at 240°. After cooling, H_2O was added and the product extracted with EtOAc. The residue was methylated with CH_2N_2 to give 20 mg of **1g** as an oil. $[\alpha]_D$ = −27.3 ($CHCl_3$; *c* 0.11); lit. −28.2° [6]. IR ν_{max}^{film} cm^{-1}: 1740, 1640, 890. ^1H NMR spectrum identical to that published previously [9].

Acknowledgements—We wish to thank Mrs. T. German and Mr. F. Ramos, Herbarium of the Instituto de Biologia (U.N.A.M.) for identifying the plant material; Messrs. J. Cardenas, R. Saucedo, A. Toscano, H. Bojorquez, R. Villana and L. Velasco, for ^1H NMR, IR and MS spectra.

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Phytochemistry, Vol. 22, No. 11, pp. 2619–2620, 1983.
Printed in Great Britain.

0031–9422/83 $3.00+0.00

24-METHYLENE-25-METHYLCHOLESTEROL, A STEROL FROM THE SEEDS OF *BRASSICA JUNCEA*

TARO MATSUMOTO, SATOSHI ASANO and TOSHIHIRO ITOH

College of Science and Technology, Nihon University, 1–8, Kanda Surugadai, Chiyoda-ku, Tokyo, 101 Japan

(*Received* 14 *March* 1983)

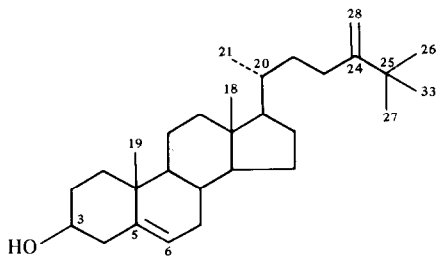
Key Word Index—*Brassica juncea*; Cruciferae; sterol; 24-methylene-25-methylcholesterol; seeds.

Abstract—A new sterol isolated from the seeds of *Brassica juncea* has been shown to be 24-methylene-25-methylcholesterol.

We have recently studied the 24-methyl-$\Delta^{5,22}$-sterol fractions, which were isolated from the seed oils of some *Brassica* and *Raphanus* species of Cruciferae plants, and demonstrated that the sterol fractions contained 10–40% of 24α-methylcholesta-5,*E*-22-dien-3β-ol in addition to its 24β-stereoisomer, 24β-methylcholesta-5,*E*-22-dien-3β-ol (brassicasterol) [1–3]. Our continuing study of the sterols of *B. juncea* seeds has now led to the isolation and characterization of a new sterol with an unusual side chain, 24-methylene-25-methylcholesterol [**1**, 24,25-dimethylcholesta-5,24(28)-dien-3β-ol].

The sterol fraction that was separated from the unsaponifiable lipid of *B. juncea* seed oil was acetylated and the resulting acetate fraction (1.8 g) was separated into four bands by silver nitrate–silica gel TLC. The fraction (44 mg) recovered from the most polar band (R_f 0.12) was subjected to reverse-phase HPLC which yielded a steryl (**1**) acetate (9 mg). GC and argentic TLC had shown that this sterol comprised 0.7% of the total sterols. The mass spectrum of **1**-acetate showed fragments at *m/z* 394 ($C_{29}H_{46}^+$, the ion of highest mass corresponding to loss of acetic acid from the molecular ion) and *m/z* 253 ($C_{19}H_{25}^+$, loss of side chain and acetic acid with 2H transfer) indicating that it was an acetate of a C_{29}-sterol with two double bonds, one of which was in the C_{10} side chain and the other probably located at C-5 [4, 5]. The side chain double bond was located either at the $\Delta^{24(25)}$- or $\Delta^{24(28)}$-position by the presence of the diagnostically significant ion of *m/z* 296 (base peak) due to a McLafferty rearrangement [4–6] involving cleavage of the C-22, C-23 bond with one H transfer from C-20 and loss of acetic acid. The 400 MHz ^{1}H NMR spectrum ($CDCl_3$) of **1**-acetate showed the following side chain signals: δ 0.965 (3H, *d*, *J* = 6.5 Hz), 1.058 (9H, *s*), 4.661 (1H, *s*) and 4.832 (1H, *s*) besides signals arising from the conventional Δ^5-3β-acetoxy sterol nucleus [7, 8] [δ 0.689 (3H, *s*, H-18), 1.022 (3H, *s*, H-19), 2.035 (3H, *s*, 3β-OAc), 4.60 (1H, *m*, $W_{1/2}$ = 28 Hz, H-3α) and 5.38 (1 H, *m*, H-6)]. The two olefinic singlets at δ 4.661 and 4.832, together with the diagnostic IR absorption at ν_{max} 895 cm^{-1} [9], indicated that the side chain double bond at C-24 must be oriented to C-24 (28) as the terminal methylene group [8, 10]. The *t*-butyl signal deshielded to δ 1.058 showed the presence of an additional methyl group at C-25 which is linked to the double bond [10, 11]. The remaining methyl doublet (δ 0.965) was then ascribed to the C-21 methyl substituent. The 20*S*-configuration is unlikely since this stereochemistry shifts the C-21 signal to the higher-field [12]. Thus the new sterol has a structure 24-methylene-25-methylcholesterol (**1**).

Chromatographic and GC/MS results have shown that **1** also occurs as a minor sterol constituent in the seeds of



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