

OXIDIZED KAURENE DERIVATIVES FROM LEAVES OF *SOLIDAGO MISSOURIENSIS* AND *S. RIGIDA*

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Key Word Index—*Solidago missouriensis*, *S. rigida*; Compositae; kaurenoic acids; kauranol.

Abstract—Oxidized kaurene derivatives were isolated from the leaves of *Solidago missouriensis* and *S. rigida* and identified as kauran-16 β -ol, kaur-16-en-19-oic acid and 7 β -hydroxykaur-16-en-19-oic acid. The structure of the latter compound was determined by X-ray crystallographic analysis of its methyl ester.

INTRODUCTION

Solidago missouriensis L. is a perennial herb native to the tall grass prairies of North America. This plant is one of 10 in experimental gardens which are being used to investigate the effects of secondary metabolite diversity upon colonization of the communities by insects [Morrow, P. A., Tonkyn, D. and Le Quesne, P. W., unpublished results]. Previous work on secondary metabolites of the closely related *S. gigantea* Ait. has involved the isolation of labdane derivatives, such as solidagoic acid **1** from the roots of *S. gigantea* Ait. var. *serotina* Cronq. [1].

RESULTS AND DISCUSSION

We have investigated the secondary metabolites of the leaves of *S. missouriensis* Nutt. and now report the isolation of three kaurene derivatives, (–)-kaur-16-en-19-oic acid (**2**), 7 β -hydroxy-(–)-kaur-16-en-19-oic acid (**3**) and (–)-kauran-16 β -ol (**4**). The structure of compound **3** was determined by X-ray crystallographic analysis of the methyl ester, since its mp was somewhat higher than that reported in the lit. [2]. Previously, this compound had been obtained by microbiological (*Cunninghamella blakesleeana*) transformation of (–)-kaur-16-en-19-oic acid [2].

Single-crystal X-ray diffraction of the methyl ester gave the relative configuration and solid state conformation of the molecule. Figure 1 has been drawn in accord with the known configuration of all other (–)-kaurane diterpenes

and, therefore, the stereostructure shown most likely represents the absolute configuration of the molecule.

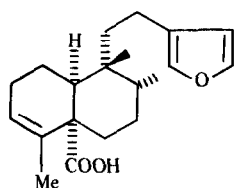
Rings A–C all have chair conformations but there is a marked increase in the deviation from ideality in the order A < B < C. The A ring is nearly ideal with an endocyclic torsion angle range of 53.1–55.6°; ring B embodies a range of 47.8–62.7° while the range for ring C is 38.4–71.0°. These distortions serve to relieve steric interactions which are potentially most severe on the α -side of the molecule. The C-20–C-19, C-20–C-14 and C-20–C-12 separations are all greater than 3.0 Å (3.02, 3.34 and 3.43 Å, respectively).

The puckering about C-4 in ring C (dihedral angles ca 70.5°) is necessitated by the bridging of the 1,3-diaxial substituents on C-8 and C-13 and induces a flattening at the C-11 end of the ring (dihedral angles ca 40.1°). Even these deformations do not separate C-11 more than 3.0 Å from the atoms in the bridge (C-11–C-16, 2.89 Å and C-11–C-15, 2.92 Å).

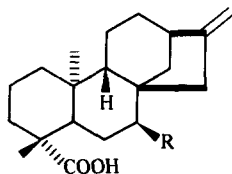
Ring D approximates to an envelope form which, while quite puckered (sum of endocyclic dihedral angles being 148.4°), is very symmetrical.

The bond lengths and valency angles all lie within accepted ranges. The mean of the bonds between the tertiary and quaternary carbons is 1.559 Å with the C-9–C-10 bond, at 1.570 Å, being the longest. As expected, the C-8–C-9–C-10 and C-9–C-11–C-12 angles are large (116.6° and 115.6°, respectively) due to the flattening about C-9 and C-11.

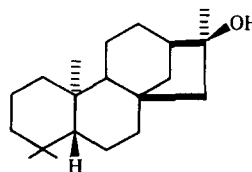
Molecules pack in layers in the y-direction. Within layers molecules are held together by hydroxyl–carbonyl



1



2 R = H
3 R = OH



4

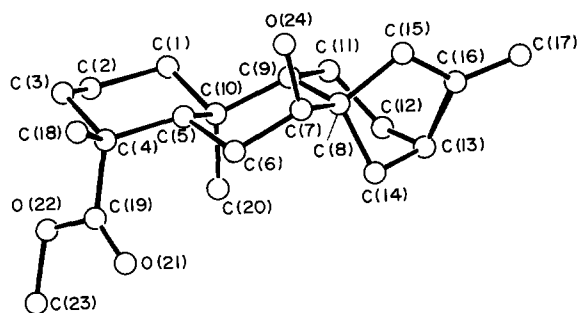


Fig. 1. A perspective view of methyl-7 β -hydroxy-(−)-kaur-16-en-19-oate, with the numbering scheme; hydrogen atoms have been omitted for clarity.

Table 1. Fractional atomic coordinates ($\times 10^4$) for the non-hydrogen atoms, with estimated s.d.s. in parentheses

Atom	x/a	y/b	z/c
C-1	2186 (3)	1657 (1)	−494 (4)
C-2	2423 (4)	972 (2)	−199 (4)
C-3	1754 (3)	725 (1)	1412 (4)
C-4	1966 (2)	1083 (1)	3175 (4)
C-5	1724 (2)	1779 (1)	2800 (3)
C-6	1734 (3)	2202 (1)	4467 (4)
C-7	1150 (2)	2811 (1)	4033 (4)
C-8	1765 (2)	3149 (1)	2489 (4)
C-9	1926 (2)	2717 (1)	823 (4)
C-10	243 (2)	2057 (1)	1208 (4)
C-11	2532 (3)	3075 (2)	−733 (5)
C-12	3489 (3)	3522 (2)	−157 (6)
C-13	3166 (3)	3887 (2)	1512 (7)
C-14	2936 (3)	3442 (1)	3088 (5)
C-15	1076 (3)	3730 (1)	1884 (4)
C-16	1980 (3)	4186 (1)	1247 (5)
C-17	1785 (4)	4752 (2)	655 (8)
C-18	1104 (3)	838 (1)	4622 (5)
C-19	3180 (2)	972 (1)	3975 (4)
C-20	3769 (2)	2059 (1)	1563 (4)
O-21	3561 (2)	1227 (1)	5301 (3)
O-22	3784 (2)	538 (1)	3093 (3)
C-23	4959 (3)	425 (2)	3754 (6)
O-24	−52 (2)	2683 (1)	3603 (3)

hydrogen bonds (O—O, 2.96 Å, OH—O, 171.7°). The only approach between layers less than 4.0 Å is a C-13—C-23 separation of 3.97 Å. Fractional atomic coordinates are shown in Table 1.

A preliminary study of the chemical constituents of the leaves of *S. rigida* L. has also led to the isolation of (−)-kaur-16-en-19-oic acid (2).

*All crystallographic calculations were carried out on a VAX 11/780 computer. The principal programs used were: FMLS, anisotropic full-matrix least-squares refinement, Ganzel, P. L., Sparks, R. A. and Trueblood, K. N., UCLA, modified by McPhail, A. T., Duke University; MULTAN 80, for description see Germain, G., Main, P. and Woolfson, M. M. (1970) *Acta Crystallogr.* B26, 274; ORTEP, crystallographic illustration programs, Johnson, C. K., Oak Ridge, ORNL-3794.

These studies are, as far as we are aware, the first analyses of secondary metabolites of the above-ground parts of *S. missouriensis* and *S. rigida*. The potential ecological significance of these metabolites is being explored further.

EXPERIMENTAL

Mps are uncorr. Low resolution MS were determined on a Nuclide 12-90-G mass spectrometer. ^1H NMR spectra were obtained with a Varian T-60 spectrometer operating at 60 MHz; in all cases TMS was used as an int. standard. ^{13}C NMR spectra were obtained with a JEOL FX-60Q instrument operating at 15 MHz. Petrol refers to the fraction of bp 35–60°. For the CC, silica gel 40 (70–230 Mesh ASTM) was used.

Plant material. Leaves of *S. missouriensis* and *S. rigida* were collected in late September from the Cedar Creek Natural History area near Minneapolis, Minnesota, U.S.A.

Isolation, purification and identification of 2–4 from S. missouriensis Nutt. Dried milled leaves (300 g) were thoroughly extracted with petrol. The petrol extract was concd to a gum (15.5 g) which was chromatographed on a silica gel column packed in petrol and eluted with petrol- CHCl_3 -EtOAc mixtures of increasing polarity. Elution with 15% CHCl_3 -petrol furnished compound 2. Compounds 3 and 4 were eluted with EtOAc-MeOH (4:1) and EtOAc, respectively.

(−)-Kaur-16-en-19-oic acid (2). Yield: 70 mg. Mp 169–170°, 185–190°; MS m/z (rel. int.): 302 (100), 287 (45), 259 (70), 243 (35), 213 (35); ^1H NMR (CDCl_3): δ 10.3 (1H, br m, COOH), 4.73 (2H, q, CH_2), 2.70 (1H, br m, CHC), 1.20 (3H, s, Me), 0.93 (3H, s, Me); ^{13}C NMR (CDCl_3): δ 184.5 (COOH), 155.7 (CCH_2), 102.9 (CCH_2) (cf. ref. [3]).

(−)-Kauran-16 β -ol (4). Yield: 20 mg. Mp 210–211°; MS m/z (rel. int.): 290 (20), 275 (18), 272 (100), 257 (60), 232 (75); ^1H NMR (pyridine- d_5): δ 4.53 (1H, br s, COH), 1.50 (3H, s, CMe), 0.98 (3H, s, Me), 0.92 (3H, s, Me); ^{13}C NMR (pyridine- d_5): δ 78.3 (COH) (cf. ref. [4, 5]).

7 β -Hydroxy(−)-kaur-16-en-19-oic acid (3). Yield: 25 mg. Mp 248–254°; MS m/z (rel. int.): 318 (10), 300 (100), 285 (20), 272 (15), 255 (28); ^1H NMR (pyridine- d_5): δ 9.10 (1H, br s, COOH), 4.36 (2H, br s, CH_2), 0.88 (3H, s, Me), 0.65 (3H, s, Me); ^{13}C NMR (pyridine- d_5): δ 180.8 (COOH), 156.6 (CCH_2), 103.8 (CCH_2), 76.9 (CHOH) (cf. ref. [2]). The methyl ester, prepared with CH_3N_2 , had mp 174–177°; MS m/z 322 [M^+]; ^1H NMR (CDCl_3): δ 4.83 (1H, s, CCH), 4.80 (1H, s, CCH), 3.65 (3H, s, COOMe), 3.63 (1H, s, CHOH), 1.17 (3H, s, Me), 0.85 (3H, s, Me).

Isolation of (−)-kaur-16-en-19-oic acid from S. rigida. Extraction of the dried leaves of this plant and chromatographic fractionation as above yielded (−)-kaur-16-en-19-oic acid as the major secondary metabolite, identified by comparison with authentic material derived from *S. missouriensis*.

Crystal data for methyl-7 β -hydroxy(−)-kaur-16-en-19-oate. $\text{C}_{21}\text{H}_{32}\text{O}_3$, $M = 333.25$. Orthorhombic, $a = 11.399(1)$, $b = 21.677(3)$, $c = 7.385(1)$ Å, $U = 1824.8(3)$ Å 3 , $Z = 4$, $D_c = 1.210$ g cm $^{-3}$, $F(000) = 728$. Cu-K α radiation ($\lambda = 1.5418$ Å), $\mu = 6.3$ cm $^{-1}$. Space group $P2_12_12_1$ (D_2^2) by systematic absences: $h00$ when $h \neq 2n$, $0k0$ when $k \neq 2n$ and $00l$ when $l \neq 2n$.

*Crystallographic measurements.** Cell dimensions were determined by least-squares refinement of 15 reflections, well-separated in reciprocal space. An octant of data was measured from $4^\circ \leq 2\theta < 130^\circ$ using a Syntex P2 $_1$ automated diffractometer in the $\theta/2\theta$, variable speed scanning mode. Two reflections measured periodically showed no significant variation in intensity. Of the 1573 reflections collected, the 1443 reflections with $I \geq 2\sigma(I)$ were corrected for Lorentz and polarization effects; an

empirical absorption correction, determined by a ψ scan, was also applied.

Structure analysis. Structure soln was effected by direct phasing methods using MULTAN 80. After several cycles of full-matrix least-squares refinement of the non-H atoms a difference Fourier synthesis was calculated to locate the H atom positions. Further adjustment of positional and thermal parameters (anisotropic O, C; isotropic H) led to convergence at $R = 0.0376$. Atomic scattering factors for O and C were taken from ref. [6]; those for H were from ref. [7].

In the least-squares refinement the function minimized was $\Sigma w\Delta^2$ and the wts were defined by $w^{1/2} = 1$ for $|F_0| < 7.6$ and $w^{1/2} = 7.6/|F_0|$ for $|F_0| > 7.6$.

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