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CRYSTAL STRUCTURE OF SANDARACOPIMARIC ACID, A LIPOXYGENASE INHIBITOR FROM *JUNIPERUS PHOENICEA*

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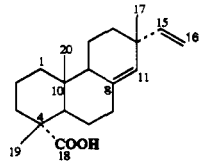
ABSTRACT.—The structure of sandaracopimaric acid [**1**], isolated from a non-polar extract of *Juniperus phoenicea*, has been determined by X-ray crystallographic analysis. This molecule was also found to be an inhibitor of soybean 15-lipoxygenase with an IC_{50} value of 0.65 mM.

Sandaracopimaric acid [**1**] is a diterpene acid of plant origin. It has been isolated from *Juniperus phoenicea* L. (Cupressaceae) and from other species (1,2).

Because arachidonic acid metabolites are important mediators of inflammation, especially the products of the lipoxygenase pathway (3,4), we used lipoxygenase inhibition as a test for several *J. phœnicea* extracts. This test allowed us to purify and characterize **1** as the agent responsible for the inhibition of lipoxygenase activity in a petroleum ether extract. The crystal structure of **1** has been determined to establish its conformation as part of a program to correlate the biological activity of lipoxygenase inhibitors and cytotoxic agents with their molecular structures (5–7).

In this study, we have examined the effects of **1** on lipoxygenase activity, and on the growth of human gastric tumor (HGT) (8) and human breast cancer (MCF-7) cell lines. Our aim was to evaluate compound **1** in order to assess the structural requirements for its pharmacological activity.



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In an effort to find naturally occurring anti-lipoxygenase compounds, organic and aqueous extracts of the plant were screened for their ability to inhibit lipoxygenase activity. Among them, the petroleum ether extract was deemed to be active, and from this we purified and characterized the active principle, sandaracopimaric acid [**1**]. The extraction of the dried leaves of *J. phoenicea* by petroleum ether yielded a residue which was subjected to chromatographic purification, affording a MeOH-soluble component. The subsequent crystallization of compound **1** was accomplished by evaporation of added MeOH at room temperature. Compound **1** was identified as sandaracopimaric acid on the basis of the comparison of its physical and spectral data with literature values.

The structure of **1** has been confirmed by an X-ray crystallographic analysis (Tables 1 and 2). A perspective view of **1** showing the molecular structure of two independent molecules with the numbering scheme is presented in Figure 1. Bond lengths and angles are very similar to those given in the literature for other terpenes (5,9). The two $C_{sp}{}^2$–$C_{sp}{}^2$ bond distances in the molecule are 1.32 (1) and 1.27 (1) Å; the $C_{sp}{}^3$–$C_{sp}{}^3$ bond lengths range from 1.49 (1) to 1.59 (2) Å. The $C_{sp}{}^3$–$C_{sp}{}^2$ bond distances are 1.51 (1) and 1.52 (1) Å. The terpene consists of three six-membered rings with chair

TABLE 1. Crystal Structure Data for **1**.

Empirical formula	$C_{20}H_{30}O_2$
Color; habit	White; prismatic
Crystal size (mm)	0.04×0.05×0.01
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a=7.458 (1) Å
	b=10.977 (2) Å
	c=43.292 (9) Å
Volume	3544.3 (0) Å^3
Z	8
Formula weight	302.46
Density (calcd)	1.13 g/cm^3
Absorption coefficient	5.2 cm^{-1}
F (000)	1328
Radiation	Cu Kα (λ=1.54184 Å)
Temperature (K)	296
Take-off angle	2.8
Scan type	ω –θ
Scan rate	1–20°/min in ω
w scan width, deg.	0.8+0.150 tan θ
Scan width, deg.	0.500 w width
2θ range	2–130°
Index ranges	0≤h≤8, 0≤k≤12, 0≤l≤50
Reflections measured	3507 total, 3473 unique
Solution	Direct methods
Refinement method	Full-matrix least-squares
Hydrogen atoms	Located and refined isotropically
Minimization function	w (|Fo|-|Fc|)^2
Least-squares weights	4 Fo/(Fo)
Anomalous dispersion	All non-hydrogen atoms
Reflections included	2399 with Fo>3.0σ (Fo)
Parameters refined	629
Unweighted agreement factor	0.054

conformations. Crystal cohesion is ensured by a three-dimensional network of van der Waals interactions and hydrogen bonds between the carboxylic groups of two independent molecules [O(21)...O(021)=2.599 (6); O(22)...O(022)=2.641 (6) Å].

The effect of **1** on soybean 15-lipoxygenase activity is reported in Figure 2. This terpene inhibited the enzyme at its optimal activity (pH 9) with an IC_{50} value of 0.65 mM. Lipoxygenase-dependent growth has been reported for various cancer cell lines such as neuroblastoma (10), mouse melanoma (11), and MCF-7 human breast cancer cells (12). Previous data have indicated that the lipoxygenase inhibitor BW755C suppressed the proliferation of HGT cells in a concentration-dependent manner (13). Therefore, we have studied the effects of **1** on the proliferation of both HGT and MCF-7 cells. Even though sandaracopimaric acid [**1**] was found to be an inhibitor of soybean 15-lipoxygenase, it did not inhibit the growth HGT and MCF-7 cells (data not shown). These results provide evidence that antilipoxygenase and antiproliferative effects may not necessarily be systematically correlated.

EXPERIMENTAL

PLANT MATERIAL.—Leaves of *Juniperus phoenicea* were collected near Roquemaure (Vaucluse-France) and dried. A voucher specimen (No. 103) has been deposited at the Laboratory of Pharmacognosy and Phytochemistry of the University of Limoges, France.

EXTRACTION AND ISOLATION.—A quantity (637 g) of the dried leaves of *J. phoenicea* was extracted using different solvents of increasing polarity, and 53.5 g of a light petroleum ether extract were obtained, with 15 g of this extract being used for the present investigation. The purification of sandaracopimaric acid [**1**] was then based on the use of several chromatographic columns [Si gel mplc with hexane-$CHCl_3$ (8:2) as solvent and Sephadex LH-20 using petroleum ether-$CHCl_3$ (1:1) as solvent], coupled with prep tlc on Si gel plates developed in $CHCl_3$-Me_2CO (1:1) as solvent system (R_f 0.80). Crystals of compound **1** were obtained by evaporation of MeOH at room temperature. Altogether, 300 mg of pure compound **1** were obtained from 15 g of the petroleum ether extract. The identification of sandaracopimaric acid [**1**] was performed by comparison of ^{1}H- and ^{13}C-nmr data and other properties with literature values (1, 2, 14–16).

X-RAY STRUCTURE ANALYSIS OF **1**.—A crystal of sandaracopimaric acid [**1**] was fixed in a random orientation on a glass fiber and mounted on an Enraf-Nonius CAD-4 diffractometer equipped with a graphite crystal monochromator. Cell dimensions were obtained from least squares refinement, using setting angles of 25 reflections in the 2–24 θ° range, measured by the computer-controlled diagonal slit method of centering. A total of 3507 reflections was collected, over a θ range of 2–130° using the ω-2θ technique with a variable scan width and scan range. Systematic absences indicated a space group of $P2_12_12_1$. Because of the low value of the absorption coefficient, the data were not corrected for absorption. After Lorentz-polarization correction, a total of 2399 reflections were used in the structural analysis.

The structural analysis was performed on a VAX computer using MolEN (17). The structure was solved by direct methods. A total of 40 atoms was located from an E-map and the remaining

TABLE 2. Non-hydrogen Atom Fractional Coordinates and Equivalent Isotropic Thermal Parameters for **1**, with Estimated Standard Deviations in Parentheses.

Atom	*x*	*y*	*z*	B (Å^2)
O-21	0.001 (1)	0.1399 (5)	0.3724 (1)	5.7 (1)
O-22	−0.030 (1)	0.2760 (5)	0.3359 (1)	6.0 (2)
O-021	0.057 (1)	0.3029 (5)	0.4148 (1)	6.5 (2)
O-022	−0.006 (1)	0.4412 (5)	0.3801 (1)	6.0 (2)
C-1	0.114 (1)	0.0129 (7)	0.2569 (2)	4.0 (2)
C-2	0.207 (1)	−0.0386 (8)	0.2853 (2)	4.7 (2)
C-3	0.182 (1)	0.0447 (8)	0.3128 (2)	4.4 (2)
C-4	−0.019 (1)	0.0656 (6)	0.3207 (2)	3.6 (2)
C-5	−0.120 (1)	0.1091 (6)	0.2910 (2)	3.1 (1)
C-6	−0.320 (1)	0.1309 (7)	0.2963 (2)	3.6 (2)
C-7	−0.401 (1)	0.1985 (7)	0.2687 (2)	3.9 (2)
C-8	−0.354 (1)	0.1454 (6)	0.2378 (2)	3.4 (2)
C-9	−0.158 (1)	0.1130 (6)	0.2339 (2)	3.2 (1)
C-10	−0.088 (1)	0.0328 (6)	0.2610 (2)	3.1 (1)
C-11	−0.473 (1)	0.1347 (7)	0.2155 (2)	4.0 (2)
C-12	−0.436 (1)	0.0974 (7)	0.1824 (2)	4.2 (2)
C-13	−0.235 (1)	0.1156 (8)	0.1765 (2)	4.6 (2)
C-14	−0.118 (1)	0.0594 (9)	0.2017 (2)	5.0 (2)
C-15	−0.534 (1)	0.1857 (8)	0.1619 (2)	5.5 (2)
C-16	−0.656 (2)	0.165 (1)	0.1421 (2)	8.0 (3)
C-17	−0.491 (2)	−0.0406 (8)	0.1769 (2)	6.2 (2)
C-18	−0.020 (1)	0.1676 (7)	0.3448 (2)	3.9 (2)
C-19	−0.097 (1)	−0.0508 (7)	0.3357 (2)	4.8 (2)
C-20	−0.183 (1)	−0.0927 (7)	0.2609 (2)	4.0 (2)
C-001	0.375 (1)	0.5426 (8)	0.4796 (2)	4.4 (2)
C-002	0.402 (1)	0.6024 (9)	0.4480 (2)	5.1 (2)
C-003	0.307 (1)	0.5283 (8)	0.4231 (2)	4.9 (2)
C-004	0.102 (1)	0.5154 (7)	0.4290 (2)	3.9 (2)
C-005	0.072 (1)	0.4671 (7)	0.4630 (2)	3.6 (2)
C-006	−0.126 (1)	0.4521 (8)	0.4706 (2)	4.6 (2)
C-007	−0.144 (1)	0.3737 (9)	0.4998 (2)	5.4 (2)
C-008	−0.037 (1)	0.4235 (7)	0.5259 (2)	4.1 (2)
C-009	0.157 (1)	0.4509 (7)	0.5181 (2)	3.7 (2)
C-010	0.175 (1)	0.5355 (7)	0.4890 (2)	3.4 (2)
C-011	−0.105 (1)	0.4344 (9)	0.5541 (2)	5.6 (2)
C-012	−0.008 (2)	0.4803 (9)	0.5823 (2)	6.4 (2)
C-014	0.263 (1)	0.4927 (9)	0.5472 (2)	5.6 (2)
C-018	0.043 (1)	0.4130 (7)	0.4064 (2)	4.4 (2)
C-019	0.005 (1)	0.6338 (8)	0.4210 (2)	5.0 (2)
C-020	0.105 (1)	0.6665 (6)	0.4968 (2)	4.2 (2)
C-013	0.154 (2)	0.557 (1)	0.5718 (2)	7.8 (3)
C-016	0.047 (3)	0.271 (1)	0.6006 (4)	15.2 (6)
C-015	0.044 (2)	0.377 (1)	0.6033 (2)	9.8 (4)
C-017	−0.136 (2)	0.572 (1)	0.6001 (3)	9.8 (4)

atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were located and their positions and isotropic thermal parameters were refined. The structure was refined in full matrix least squares and the final unweighted R value was 0.054. Values for positional parameters and their estimated standard deviations for sandaracopimaric acid [**1**] are available.[1]

[1]Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

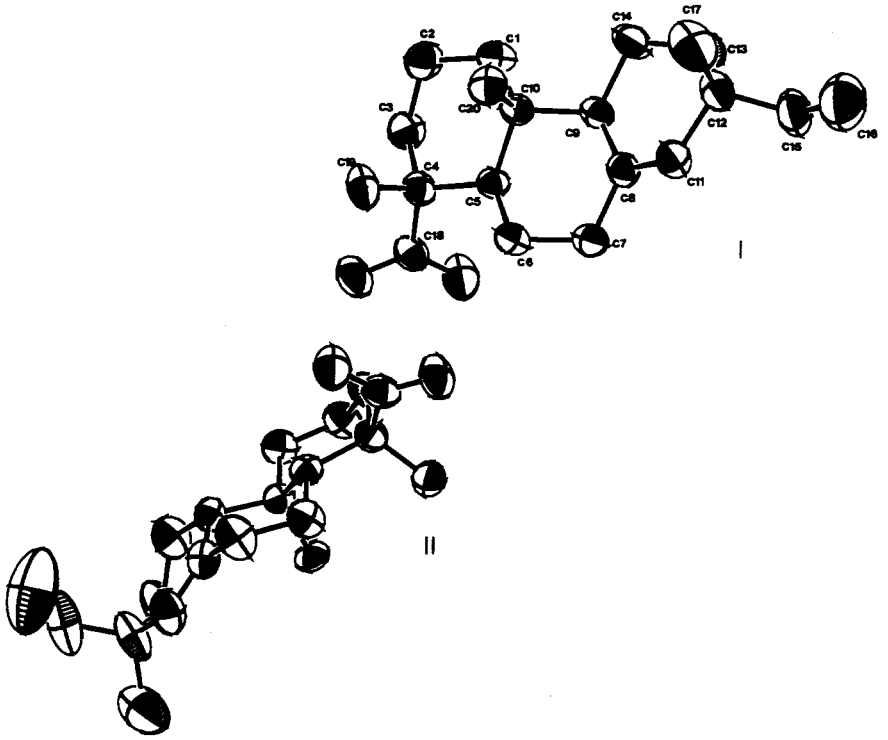

FIGURE 1. A perspective view of two independent molecules of sandaracopimaric acid [**1**].

SOYBEAN LIPOXYGENASE ASSAY.—The standard assay mixture contained the enzyme soybean 15-lipoxygenase (from Sigma) and the reaction was started by addition of linoleic acid (Sigma). Inhibition experiments were run by measuring the loss of activity of 15-lipoxygenase (0.11 μM) in the presence of various concentrations of diterpene **1**. Lipoxygenase activity was determined spectro-

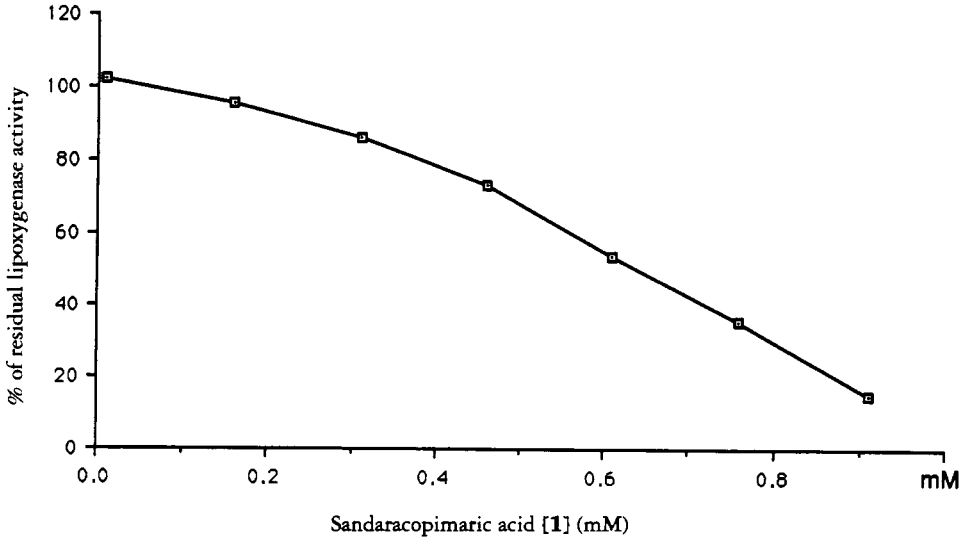

FIGURE 2. Effects of sandaracopimaric acid [**1**] on in vitro soybean 15-lipoxygenase activity (pH 9). Results are expressed as percentages of residual activity, and represent the mean of three independent experiments in triplicate. Standard deviations were less than 5% in all cases.

photometrically by monitoring the 234 nm absorbance of 13-(*S*)-hydroperoxy-*cis*-9-*trans*-11-octadecadienoic acid [13(*S*)-HPODE] (ϵ max=25000 M^{-1} cm^{-1}), formed from linoleic acid (71 μM) at 20° in 1 ml of 0.2 M borate buffer (pH 9).

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