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(3*S*,4*S*,5*R*)-3,4,5-TRIHYDROXY-1-CYCLOHEXENE-CARBOXYLIC ACID FROM *SEQUOIADENDRON GIGANTEUM*

HANS GEIGER,* SAAD EL-DESSOUKI and TASSILO SEEGER

Fachrichtung Botanik, Universitaet des Saarlandes, Postfach 151150, D-66041 Saarbruecken, Germany

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Key Word Index—*Sequoiadendron giganteum*; Taxodiaceae; (3*S*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexenecarboxylic acid; epimer of shikimic acid.

Abstract—From the leafy lateral branchlets of *Sequoiadendron giganteum*, (3*S*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexenecarboxylic acid has been isolated. Its structure was proved spectroscopically.

INTRODUCTION

Some 35 years ago, the occurrence of an isomer of shikimic acid in *Sequoiadendron giganteum* was reported [1]. This report has, however, never been substantiated. During a study of the flavonoids of *S. giganteum*, we have also isolated the non-phenolic acids, which turned out to be a mixture of shikimic acid and an isomer thereof [2, 3].

RESULTS AND DISCUSSION

The separation of the two components was achieved by fractional recrystallization of the methyl esters. This gave, besides methyl shikimate, an isomeric methyl ester, the melting point and optical rotation of which agreed with published data of a methyl *epi*-shikimate that had been obtained along with methyl shikimate by reduction of methyl dehydroshikimate with sodium borohydride [4]. Since no authentic material was available for direct comparison, the structure of our ester had to be confirmed by its NMR spectra (Table 1). *trans*-Diaxial coupling between H-3 and H-4, as well as H-4 and H-5 ($J_{3,4} = 7.8$ Hz and $J_{4,5} = 9.8$ Hz) demonstrated that H-3, H-4 and H-5 are situated in the Haworth projection at alternating sides of the ring plane and that the conformation is half-chair with H-3, H-4, H-5 and H-6β in axial and the hydroxyl groups, as well as H-6 α, in equatorial positions. Thus, the ester from *S. giganteum* has the same relative and, because of an identical optical rotation, also the same absolute configuration as the synthetic product; it is therefore (3*S*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexenecarboxylic acid methyl ester (**2**). This ester **2** was previously named 5-*epi*-shikimic acid methyl ester [4], but this name is, however, no longer tenable, since in the meantime the numbering system for shikimic acid has been changed by IUPAC rules (see Ref. [5] and references quoted therein). These rules require, that the double bond is located between C-1 and C-2 as in the systematic name of **2**, and not between C-1 and C-6 as the old numbering system for quinic acid had required after Fischer and Dangschat had proved the location of the double bond in shikimic acid [6].

Free (3*S*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexenecarboxylic acid (**1**) was hitherto unknown; it was prepared by alkaline hydrolysis of the ester **2**. The acid **1** as well as its ester **2** can be separated from shikimic acid and its methyl ester by TLC on silica gel impregnated with boric acid. The acids and their esters can be visualized selectively by oxidation with KIO_4 to aconitic-1,5-dialdehyde or its ester, respectively, and condensation of these dialdehydes with aniline or *N*-methylaniline to characteristically-coloured polymethine dyes (Table 2). Using this method it will be possible to examine if the occurrence of **1** in *S. giganteum* is unique or whether it occurs more widely.

EXPERIMENTAL

Plant material. This consisted of leafy lateral branchlets of *S. giganteum* (Lindl.) Buchholz. It was collected in November 1972 in the Botanical Garden of the University of Stuttgart-Hohenheim and identified by Prof. B. Frenzl, the head of the garden.

TLC. TLC plates were coated with a slurry of Kieselgel G that was made with 0.1 M $B(OH)_3$ instead of H_2O [8]. Solvent 1: *n*-BuOH–Me_2CO–H_2O (4:5:1); solvent 2: EtOH–Me_2CO–H_2O (4:5:1). Spray reagents: a, satd aq. KIO_4; b, 1% aq. $NaHSO_3$; c, 1% aniline or *N*-methylaniline in MeOH. Sprays are applied in the sequence a, b and c, and the plates are allowed to dry between the

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*Author to whom correspondence should be addressed.

Dedicated to Professor H. D. Zinsmeister on the occasion of his sixtieth Birthday.

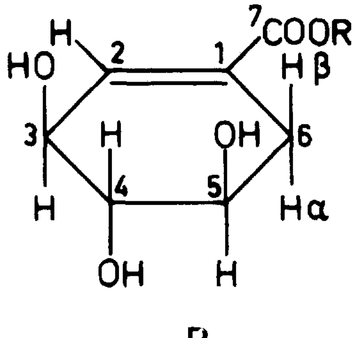
Table 1. ^{13}C and ^{1}H NMR data of compound **2** (MeOH-d_4, ambient temperature, 125 and 500 MHz, respectively). ^{1}H signals are correlated by COSY, ^{1}H- and ^{13}C-signals by the inverse technique

Position	^{13}C δ(ppm)	^{1}H δ(ppm)	Multiplicity	Coupling constants (Hz)
1	129.2	—	—	—
2	140.7	6.65	*m*(3)*	3.0 ($J_{2,6\beta}$), 1.9 ($J_{2,3}$)
3	73.2	4.15	*m*(9)*	7.8 ($J_{3,4}$), 3.8 ($J_{3,6\beta}$), 1.9 ($J_{2,3}$), 1.4 ($J_{3,6\alpha}$)
4	78.3	3.37	*dd*	9.8 ($J_{4,5}$), 7.8 ($J_{3,4}$)
5	70.3	3.64	*m*(6)*	9.8 ($J_{4,5}$), 9.8 ($J_{5,6\beta}$), 5.9 ($J_{5,6\alpha}$)
		α2.76	*ddd*	17.5 ($J_{6\alpha,6\beta}$), 5.9 ($J_{5,6\alpha}$), 1.4 ($J_{3,6\alpha}$)
6	33.6			
		β2.16	*dddd*	17.5 ($J_{6\alpha,6\beta}$), 9.8 ($J_{5,6\beta}$), 3.8 ($J_{3,6\beta}$), 3.0 ($J_{2,6\beta}$)
7	168.3	—	—	—
CH_3	52.4	3.76	*s*	—

*Values in parentheses refer to number of observable lines in each multiplet. With the aid of the ^{1}H–^{1}H COSY spectrum and the coupling constants extracted from the fully resolved signals, all three multiplets can be analysed by a first-order approach.

Table 2. TLC data of compounds **1**, **2**, shikimic acid and methyl shikimate

	hR_f		Colour with	
	Solvent 1	Solvent 2	Aniline	*N*-Methylaniline
1	34	47	Red	Orange
Shikimic acid	20	25	Red	Orange
2	86	83	Yellow	Red
Methyl shikimate	69	66	Yellow	Red



R
1: H
2: Me

sprays. Colours appeared soon after the final spray; the background becomes discoloured with time. For hR_f values and colours, see Table 2.

Isolation and identification. Dried and ground material (1.3 kg) was defatted with CH_2Cl_2 and then exhaustively extracted with an azeotropic mixt. of MeCOEt and H_2O. The extract was evapd *in vacuo* and subjected to CC on polyamide-6 with a H_2O–Me_2CO gradient as described in ref. [7]. Frs emerging before the flavonoid glycosides were freed from Me_2CO and passed over a short column containing weakly basic anion-exchange resin (Lewatite MP 60). After washing the resin with 300 ml H_2O, acids were eluted with 600 ml 25% HOAc and the eluates evapd *in vacuo*. Trituration of the residue with MeOH gave 10 g of a crystalline mixt. of **1** and shikimic acid. It had mp of 170–174° and an equiv. wt of 172 (alkalimetric). This mixt. was methylated with CH_2N_2 and the resulting esters separated by fractional recrystallization [4]. After many recrystallizations, apart from shikimic ester and a large mixed fr. *ca* 1 g of pure **2** was obtained. Colourless needles, mp 133° (lit. [4]: 133°). $[\alpha]_D^{20}$: $-14 \pm 1°$ (MeOH, *c* 2) (lit. [4]: $[\alpha]_D^{16}$: $-13.2°$ (MeOH). (Found: C, 51.2; H, 6.32. Calc. for $C_8H_{12}O_5$: C, 51.06; H, 6.43%).

(3S,4S,5R)-3,4,5-*Trihydroxy*-1-*cyclohexenecarboxylic acid* (**1**). Compound **2** (0.5 g) and 10 ml 2M NaOH were heated for 2 hr at 100°. The cooled soln was freed from Na^+ ions by ion-exchange (Dowex 50 W × 2) and evapd. Trituration of the syrupy residue with MeOH yielded **1** as tiny, white crystals, mp 181–183°. $[\alpha]_D^{20}$: $-20 \pm 1°$ (MeOH, *c* 0.8). (Found: C, 48.39; H, 5.57. $C_7H_{10}O_5$ requires: C, 48.28; H, 5.75%).

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REFERENCES

1. Plouvier, V. (1959) *C. R. Acad. Sci. Paris* **249**, 1563.
2. Geiger, H. and Buck, R. (1973) *Phytochemistry* **12**, 1176.
3. El-Dessouki, S. (1974) Dissertation, Hohenheim.
4. Grewe, R. and Jeschke, J.-P. (1956) *Chem. Ber.* **89**, 2080.
5. Haslam, E., Turner, M. J., Sargent, D. and Thompson, R. S. (1971) *J. Chem. Soc.* (*C*), 1489.
6. Fischer, H. O. L. and Dangschat, G. (1937) *Helv. Chim. Acta* **20**, 705.
7. Beckmann, S. and Geiger, H. (1968) *Phytochemistry* **7**, 1667.
8. Stahl, E. (1967) *Dünnschichtchromatographie, ein Laboratoriumshandbuch* (2. Auflage), p. 770. Springer, Berlin.