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DAUCOGLABRIN, A TRIESTER PHENYLPROPANOID FROM DAUCUS GLABER (FORSSK) TELL.

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ABSTRACT

Daucoglabrin is a new phenyl propanoid isolated from the aerial parts of Daucus glaber (Forssk)Tell. Its structure was established, based on chemical and spectral data as : 2-[2-hydroxy-2-methyl-3-chlorobutanoyloxy]-1-[2-hydroxy-2-methyl-3-(3-methyl-2-butenoyloxy)-butanoyloxy]-1-(3-methoxy-4,5-methylenedioxy phenyl) propane.

INTRODUCTION

Daucus glaber (Forssk) Tell (D. littoralis Sibth et Sm. Var. Forsskahli Boiss.) is a decumbent umbelliferous herb, with short glabrous stem and much branched at the base[1,2]. It grows well in sand dunes and sandy sea shores in the Northern region of the Nile Delta and flowers from March to early May.

The current literature is devoid of any information concerning the chemical constituents of this species following its present as well as its old name. However, the related variety, Daucus littoralis Sibth and Sm. was investigated for its fruit volatile oil[3] and flavonoids[4] as well as leaf and stem flavonoids[5].

The present investigation is concerned with the isolation and structural elucidation of a new phenyl propanoid compound 1, for which the name daucoglabrin is proposed.

EXPERIMENTAL

Plant Material :

The aerial parts (stem and leaf, collected at the flowering stage in May, 1985, from Balteem, Kafr El-Shiekh) were air-dried, powdered, sieved with sieve No. 10, and stored in dark brown bottles till used. Fresh plant material was reserved in 5% methanol in aqueous glycerol for botanical study. The plant material was identified by Dr. I.Mashaly, at the Royal Botanic Garden Kew, London, England.

Extraction and Isolation Procedure :

The powdered plant material (1.5 kg) was extracted by refluxing 3 times with methanol (6 l. each) for 5 hr (each extraction). The combined methanol extract was concentrated to about 150 ml, diluted with an equal volume of water, then extracted with light petroleum 40-60°, (4 x 300 ml) followed by chloroform (4 x 200 ml). After evaporation of the solvents, the light petroleum and chloroform extracts yielded 36 g and 12 g of dark green residues respectively. The chloroformic residue was reserved for future investigation of its bitter-tasting constituents.

The light petroleum extract (25 g) was chromatographed over silica gel (550 g, E. Merck) using light petroleum (900 ml), light petroleum-chloroform (9 : 1 v/v, 500 ml), light petroleum-chloroform (3:1 v/v, 650 ml), chloroform (10 fractions, 100 ml each), for elution. On concentrating the fraction eluted with light petroleum-chloroform, 3:1, a solid substance was deposited. After filtration and repeated crystallization from ether-light petroleum, white fine prisms of 1 were obtained (55 mg), m.p 118-120°.

Instruments :

IR : KBr, Pye Unicam 1000, MS : Varian MAT 44S, 70 eV, direct inlet, ^{1}H-NMR : in $CDCl_3$, TMS as internal standard, Bruker WM 400, ^{13}C-NMR in $CDCl_3$, Bruker WH 270, operating at 67.9 MHz.

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The following spectroscopic data were revealed by 1 (refer to the numbering system assigned[9]).

IR : (cm^{-1}) : 3525, 3500, 1748, 1727, 1702, 1610, 1515.

Mass 70 ev : m/z (rel. int.):

560(10, M + 2), 558 (27, M^+), 427 (1),
345 (2), 343 (6), 306 (2), 282 (2).
279 (7), 209 (22), 208 (35),
199 (4), 192 (44), 181 (8),
171 (16), 165 (14), 83 (100), 55 (25).

Accurate mass: measured 558.186

($C_{26}H_{35}O_{11}Cl$) ; Calculated 558.187

^{1}H-NMR : (Table 1)

^{13}C-NMR: (Table 2)

RESULTS AND DISCUSSION

Daucoglabrin occurs as white fine prisms, m.p. 118-120°, soluble in chloroform and methanol sparingly soluble in light petroleum and practically insoluble in water. Its IR spectrum revealed absorption bands for hydroxyl at 3500-3525, three carbonyls at 1748, 1727, 1702 and aromatic residue at 1610,1515 cm^{-1}. The hydroxyl group (s) must be tertiary because the substance was recovered unchanged on treatment with acetic anhydride-pyridine. The three carbonyl functions were proved to be esters from ^{13}C-NMR which showed three singlets at 174.59, 173.59 and 164.94 ppm (Table 2).

The ^{1}H-NMR (Table 1) revealed only two nonequivlent aromatic protons at δ 6.47 (d,J=1.5 Hz) and 6.41(d,J=1.5 Hz) characteristic for meta-positioned protons.

The ring substituents were represented by a methoxyl group (3.9, 3H, s), methylene dioxy group (5.93, 2H, s) and an acylated propane diol moiety. as judged from signals at δ 5.09 (3H, d,J = 7.0 Hz).This phenyl propane moiety was fully confirmed by the ^{13}C-NMR (Table 2).

On the other hand, the presence of one chlorine atom in the structure was realised from the mass spectral data (see experimental).
A molecular ion peak at $M^{+\cdot}$/z 558 was established. On careful examination, the relative intensity of the M+2 ($m^{+\cdot}$/z 560) was nearly 37% of that of the M^{+}, a case characteristic for the presence of one chlorine atom in the molecule[6].

Accurate mass measurements gave a molecular ion peak at 558,186 which is almost consistent with a molecular formula $C_{26}H_{35}O_{11}Cl$, calculated 558.187.

The established structure of the phenyl propane diol moiety accounts for 11 carbons and we are now left with a total of fifteen carbon moieties which contribute the three ester linkages. In other words, we have three acid residues each assumed to be of 5-carbon hemiterpene type of common occurrence in this family. One acid esterified one of the diol hydroxyls and the second acid esterified the other hydroxyl and being itself esterified by a third acid.
This assumption was substantiated by the spectral data as follows :

One of the acids must be senecioic acid (3-methyl-2-butanoic acid) as clearly indicated by both ^{1}H-NMR signals viz.two allylic methyls at 1.77 and 2.02 (broad singlets) and a vinylic proton at δ 5.16 (broad singlet) and the mass spectral data viz. base ion peak at m/z 83 assigned for-$\overset{O}{\overset{\|}{C}}$-CH=C$(CH_3)_2$ and m/z 55(26%, 83-CO) . Therefore senecioic acid either directly acylated one of the phenyl propane diol hydroxyls or a hydroxyl of one of the other acids. This point was solved by considering the first significant mass fragments at m/z 343 (6) assigned for M-$C_{10}H_{15}O_5$, i.e. the loss of an acyl radical represented by senecioyl ester of another five carbon dihydroxy acid .

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Since the free hydroxyl group is tertiary as reported before, this acid must be a dihydroxy-methyl butanoic acid (DMB).

The m/z 343 fragment should, therefore, comprise the phenyl propane moiety acylated with the third acid. The latter should contain one chlorine atom and has the emperical formula $C_5H_9O_3Cl$. Similarly, since the hydroxyl group (s) are only tertiary, this acid must be a hydroxy-methyl-chlorobutanoic (HMCB). The prominant fragment at m/z 208 (35) was assigned for 343-$C_5H_7O_2Cl$.

The exact structures of the DMB and HMCB were established by ^{1}H-NMR and ^{13}C-NMR as 2,3-dihydroxy-2-methyl butanoic acid and 2-hydroxy-2-methyl-3-chlorobutanoic acid respectively as indicated in Tables (1,2).

To complete the structural elucidation of daucoglabrin, it was necessary to know the esterification sites of these acids on the phenyl propanediol. The MS data readily indicate that senecioyl-DMB is the one acylating the C-1 hydroxy of the diol system since on loosing this acid, the benzylic ion produced would be highly stabilized by the aromatic ring and thus revealing the significance of the fragment m/z 343. The total absence of a mass fragment at m/z 407 (M-$C_5H_8O_3Cl$) confirms that the position of HMCB is confined to the C-2 hydroxyl of the diol.

Finally, the relative stereochemistry at C-1 and C-2 could be infered by careful examination of the ^{1}H.NMR coupling constants of daucoglabrin.

From ^{1}H NMR of daucoglabrin, H-1 appears as doublet at δ 5.72 J=8.5 Hz), i.e. $W_{\frac{1}{2}}$ of the doublet is more than 4.0 Hz demonstrating that H-1 must be α to the next door hydrogen H-2 which must be B[6,7]. In addition ^{13}C-1 gives a signal in ^{13}C-NMR at δ 78.9 a typical shift value for a carbon carrying B-oxygen substituent[8]. The same conclusion could be reached by comparing the ^{1}H NMR data with those of two related phenyl

propane compounds viz., helmanticin (II) isolated from Thapsia villose L[9]. with erythro-configuration of the diol and laserin (III) isolated from Laser trilobum[10] with threo-configuration The coupling constant J1, 2 for the threo-configuration is 7.5 Hz which is close to that of daucoglabrin (8.5 Hz) and larger than that of the erythrodiol (4.5 Hz). Therefore, daucoglabrin has a 1,2-threo-configuration.

For further confirmation daucoglabrin was saponified with 5% KOH-MeOH. The phenyl propanediol was separated by extraction with ether followed by crystallization to yield white prisms, m.p. 109-110°, close to that reported for deacyl laserin m.p. 111°[10] and differs from that of deacyl helmanticin, m.p. 79°[9].

Therefore the proposed structure of daucoglobrin is : 2-[2-hydroxy-2-methyl-3-chlorobutanoyloxy]-1- [2-hydroxy-2-methyl-3(3-methyl-2-butenoyloxy)-butanoyloxy]-1-(3-methoxy-4,5-methylenedioxyphenyl) propane.

Chlorine-containing natural compounds in higher plants were recently reviewed by Engwild[11]. The Apiaceae contains several chlorinated coumarins where the chlorine atom in many examples is a part of the same acid moiety, 2-hydroxy-2-methyl-3-chlorobutanoic acid, as in the case of daucoglabrin.

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Table 1. ^{1}H.NMR,: δ ($CDCl_3$: 400 MHz)

Hydrogen No.	(ppm)	Multiplicity, (J)
1	5.72	1H, d, J=8.5 Hz
2	5.24	1H, dq, J=8.5, 7 Hz
3	1.09	3H, d, J=7 Hz
3′	4.96	1H, q, J=7.5 Hz
4′	1.25	3H, d, J=7.5 Hz
5′	1.39	3 H, s
7′	5.16	1H, s
9′	1.77	3H, s
10′	2.02	3H, s
3″	4.27	1H, d, J=7 Hz
4″	1.54	3H, d, J=7 Hz
5″	1.42	3H, s
2″	6.47	1H, d, J=1.5 Hz
6″	6.41	1H, d, J=1.5 Hz
-OH	3.19	S
$O-CH_2-O$	5.93	S
$-OCH_3$	3.90	(3H, S)

Table. 2. ^{13}C-NMR: δ ($CDCl_3$: 67.9 MHz

Carbon No.	(ppm)	Multiplicity	Carbon no.	(ppm)	Multiplic.
1	78.9	d	1‴	129.6	s
2	73.5	d	2‴	106.7	d
3	13.2	q	3‴	143.9	s
			4‴	135.7	s
′1	174.6	s	5‴	148.8	s
′2	76.2	s	6‴	102.1	d
′3	73.8	d			
′4	22.6	q			
′5	20.0	q			
′6	164.9	s			
′7	115.1	d			
′8	157.4	s			
′9	16.6	q			
′10	27.2	q			
″1	173.6	s			
″2	77.2	s			
″3	62.5	d			
″4	21.8	q			
″5	17.9	q			
OCH_3	56.3	q			
$O-CH_2-O$	101.6	t			

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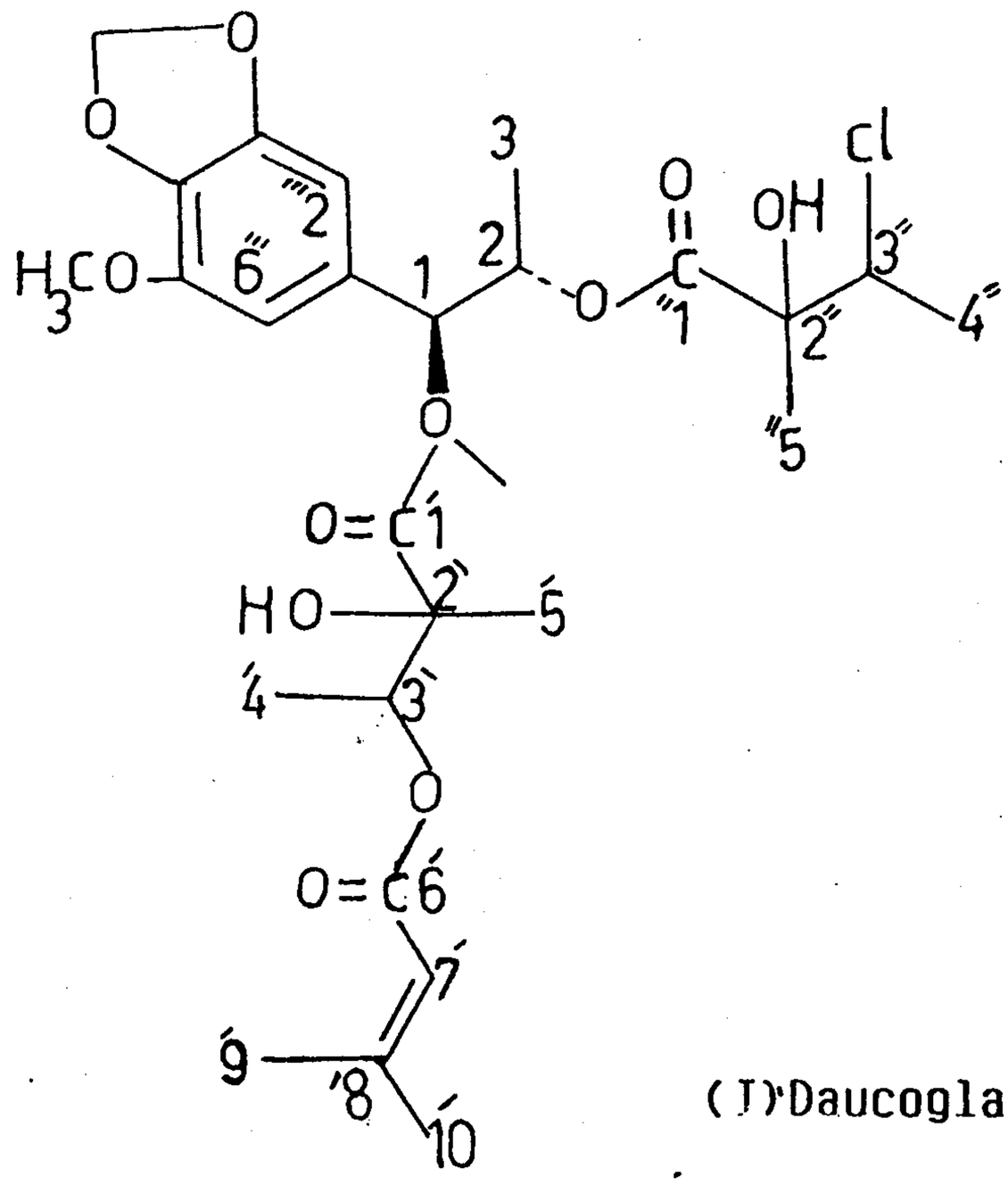
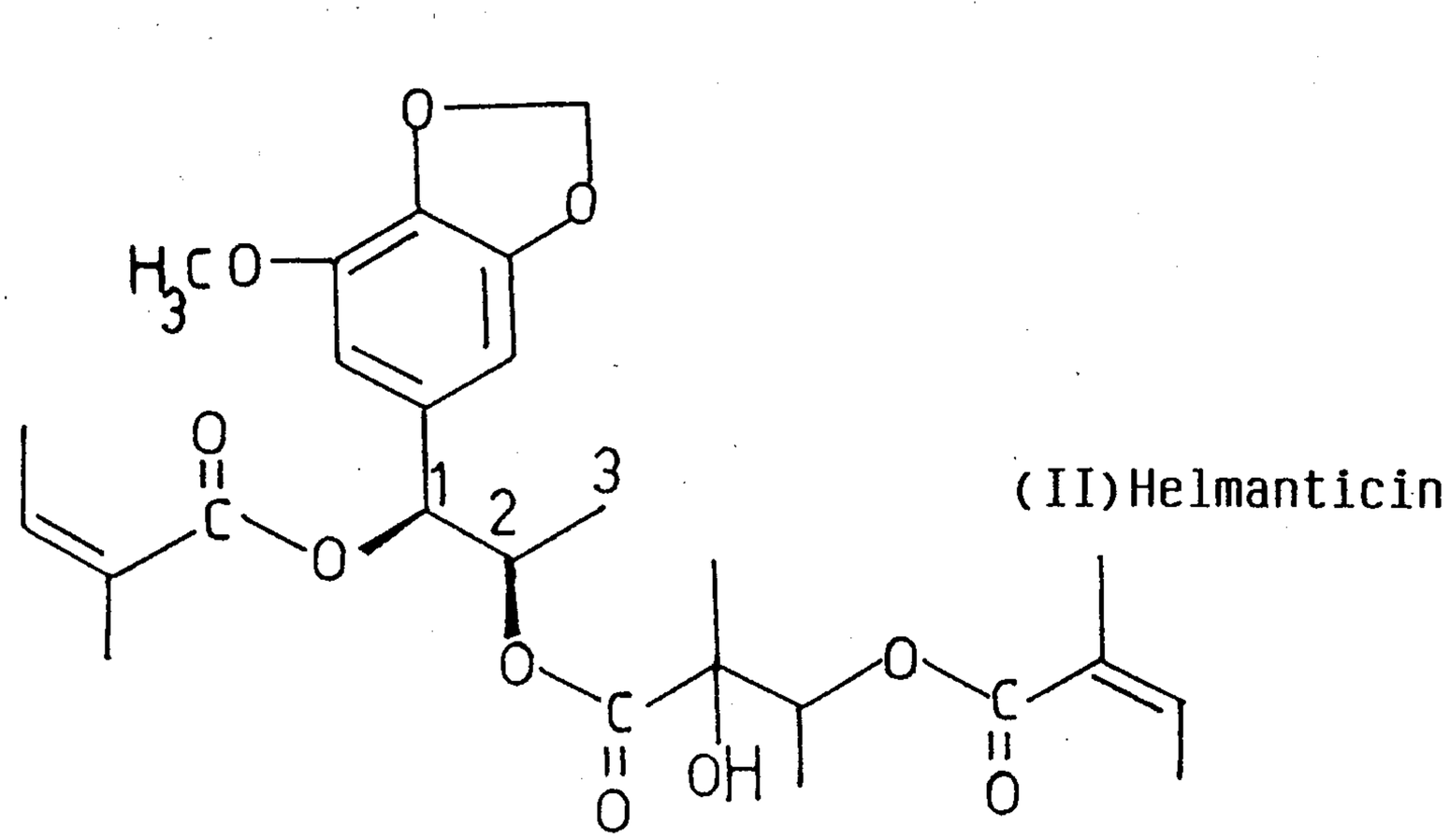
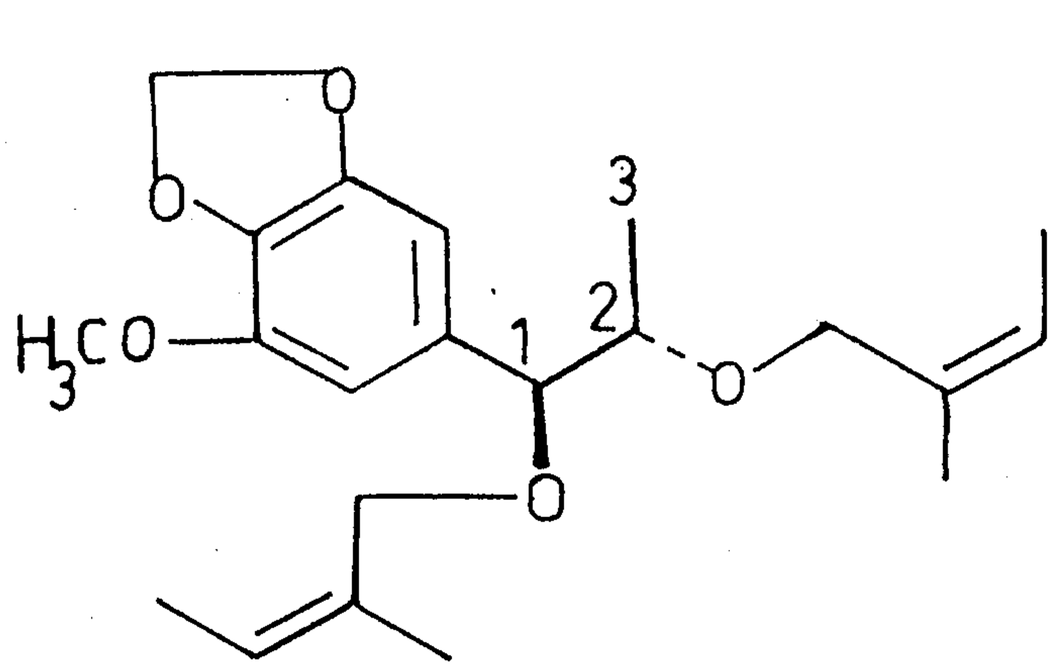


(I)Daucoglabrin



(II)Helmanticin



(III)Laserin

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داوكوجلابرين ، فينيل بروبانويد ثلاثى الأستر من نبات داوكس جلابر (فورسك) تل

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تم فصل مركب جديد سمى داوكوجلابرين من خلاصة الكحول الميثيلى لأوراق وسيقان نبات داوكس جلابر (فورسك) تل .
وأمكن استجلاء بنيته الكيمائية بالطرق الطبيعيه والكيمائيه والطيفيه المختلفه . وثبت أنه يتبع مجموعة الفينيل بروبافويد ويتميز بأنه يحتوى على ثلاثة مجموعات استيريه لثلاثة أحماض نصف تربينيه ، أحدهم يحوى ذرة كلور .

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