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THE STRUCTURE OF MEZEREIN, A MAJOR TOXIC PRINCIPLE OF DAPHNE MEZEREUM L.

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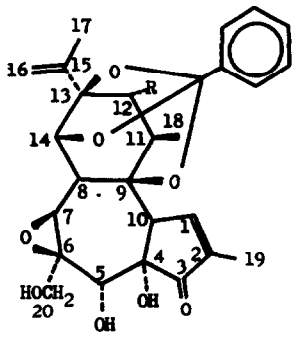
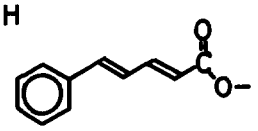
The isolation and crystallographic structure determination of a toxic principle, daphnetoxin (I) from the bark of Daphne mezereum L. and other Daphne species has recently been reported by Stout et al.[1]. The daphnetoxin isolated accounted for about 1/3 of the total toxicity of the plant material. In an independent investigation we have found the seeds of D. mezereum to contain ca. 0.1 % of a major toxic compound, mezerein (II) accompanied by small amounts (ca. 0.02 %) of daphnetoxin. (Mezerein and its hexahydro derivative produce the same type of skin lesions as daphnetoxin.)

Mezerein, $C_{38}H_{38}O_{10}$, was first obtained as an ether solvate (ca. 0.5 mole of diethyl ether); m.p. 265 - 269°d.; $[\alpha]_D^{25}$+117.5° ($CHCl_3$); λ_{max}^{EtOH} (log ε) 227 (4.24), 234 (4.29), 241 (4.27), 314 (4.60) nm; ν_{max}^{KBr} 3520, 1714, 1698, 1626, 754, 695 cm^{-1}; mass spectrum m/e 654.2482 (M^+) 157, 149, 105; NMR (see tables 1 and 2). Daphnetoxin, m.p. 194 - 195.5°, was identified by spectral data and by the formation of a diacetate (IV). NMR assignments for daphnetoxin (table 1) are based on the published structure and were confirmed by decoupling experiments.

Given the structure of daphnetoxin, available evidence shows that mezerein has structure II. The presence of a cinnamylideneacetic ester function in mezerein is indicated by the methoxide catalyzed methanolysis of mezerein under mild conditions to yield methyl 5-phenyl-2,4-pentadienoate[2] and an amorphous alcohol (III), $C_{27}H_{30}O_9$; $[\alpha]_D^{25}$+32.1° (ethanol); λ_{max}^{EtOH} (log ε) 243 (4.12, 333 (1.1) nm; ν_{max}^{KBr} 3465, 1705, 1695, 1631, 750, 695 cm^{-1}; mass spectrum m/e 498 (M^+), 149, 105. While acetic acid in pyridine converts mezerein into a diacetate (V;



I R = H

II R = 

III R = OH

Table 1.

Significant signals in the NMR spectra of daphnetoxin (I), mezerein (II), alcohol III, daphnetoxin diacetate (IV), mezerein diacetate (V), the triacetate of alcohol III (VI), mezerein mono-(p-bromobenzoate) (VII) and the dihydro derivative of III (VIII)[a,b].

Compound	H-1m	H-5s	H-8d	H-10m	H-12	H-14d	H-20A[c]	H-20B[c]
I	7.62	4.26	3.02	3.91	2.36m	4.57	3.83s	3.83s
II	7.50	4.20	3.64	3.88	5.12s	4.94	3.91s	3.75s
III	7.52	4.21	3.79	3.91	4.03s	4.84	3.79s	3.79s
IV	7.52	5.56	3.08	4.05	2.35m	4.56	4.78	3.63
V	7.55	5.56	3.73	4.05	5.15s	4.97	4.78	3.63
VI	7.55	5.42	3.74	4.05	5.13s	4.78	4.78	3.63
VII	7.51	4.42	3.73	4.10	5.23s	5.14	4.94	3.27
VIII	7.59	4.2.	3.77	3.75	4.09s	4.65	3.75s	3.75s

[a]Assignments for compounds II, III and V-VIII were made by comparison with the NMR spectra of compounds I and IV of known structure and were confirmed by decoupling experiments indicated in table 2.

[b]Apart from VII the mezerein derivatives, although chromatographically homogeneous, were not crystalline.

[c]In compounds IV-VII the C-20 protons appear as an AB-quartet with J_{AB} = 12.5 Hz (confirmed by INDOR experiments).

Table 2.

Decoupling in the NMR spectrum of mezerein[a].

Irradiated at	ppm	Observed at	ppm	Change in multiplicity	Coupling removed Hz
H-1	7.48	H-10	3.85	sharpening	< 0.5
H-1	7.48	H-19	1.74	dd → d	1.2
H-5	4.21	OH-5	4.11	d → s	3.0
H-8	3.61	H-14	4.95	d → s	2.3
H-10	3.85	H-1	7.48	sharpening	< 0.5
H-10	3.85	H-19	1.74	dd → d	2.6
H-11	2.47	H-18	1.36	d → s	7.0
H-18	1.36	H-11	2.47	q → s	7.0

[a]The mezerein derivatives (V-VIII) give similar results.

m/e 738 (M^+)), alcohol III yields a triacetate (VI; m/e 624 (M^+)) under similar conditions.

NMR data (table I) provide strong evidence that the alcohol III is 12-hydroxydaphnetoxin. Thus the protons at C-11 and C-12 in daphnetoxin give rise to a three-proton multiplet at δ 2.1 - 2.7 ppm, while in alcohol III H-11 appears alone in this region as a one-proton quartet. H-12 in alcohol III appears as a one-proton singlet at δ 4.03. A further downfield shift of this signal by 1.1 ppm in mezerein, mezerein diacetate (V) and the triacetate VI is consistent with the formulation of mezerein as 12-(cinnamylideneacetoxy)daphnetoxin (II).

Dreiding models and the published crystallographic data [1] indicate that the six membered ring (ring A) in mezerein has a twisted boat conformation. The C-12 hydroxyl group apparently has the rather hindered endo (flagpole) configuration. However, in all mezerein derivatives, the NMR signal of H-8 is shifted downfield by ca. 0.6 ppm as compared to daphnetoxin and its diacetate. This effect must be due to a strong deshielding effect exerted upon H-8 by endo C-12 substituents. The absence of detectable coupling between H-11 and H-12 is

indicative of a dihedral angle close to 90° between these protons. This suggests an exo H-12 configuration since van der Waals interaction would be expected to cause a slight flattening of ring A so that the dihedral angle between H-11 and exo H-12 will approach a right angle.

Mezerein absorbs 3 moles of hydrogen on hydrogenation over a palladium-carbon catalyst in ethanol, affording amorphous hexahydromezerein, $C_{38}H_{44}O_{10}$; mass spectrum m/e 660.2945 (M^+); λ_{max}^{EtOH} (log ε) 242 (3.70), 330 (2.0) nm. Methanolysis of hexahydromezerein yields methyl 5-phenylvalerate[3] and a dihydro-alcohol; mass spectrum m/e 500 (M^+), 149, 105. Spectral data indicate that the hydrogenation in the diterpenoid part of the molecule saturates the isopropenyl side chain. More vigorous alkaline or acid hydrolysis of mezerein leads to extensive degradation with the formation of cinnamylideneacetic acid and benzoic acid. The IR spectra of mezerein and its derivatives exhibit peaks in the 695 and 750 cm^{-1} regions indicative of a monosubstituted benzene ring. CD data for mezerein ($[\theta]_{323}$ +16000, $[\theta]_{243}$ -41000), alcohol III ($[\theta]_{343}$ +1800, $[\theta]_{245}$ -9800) and mezerein diacetate ($[\theta]_{325}$ +13000, $[\theta]_{243}$ -19000) are in agreement[4] with the proposed structure and the data reported for daphnetoxin[1].

The occurrence of mezerein and its 12-desoxy analogue daphnetoxin in a Thymelaeaceae species parallels the occurrence (as esters) of phorbol[5], 12-desoxyphorbol[6] and 16-hydroxy-12-desoxyphorbol[7] in the Euphorbiaceae. This adds further weight to the possibility of a close relationship between the Thymelaeaceae and the Euphorbiaceae which is already supported by the occurrence of daphnetoxin[1].

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