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THAPSIGARGIN, CONSTITUTION OF A SESQUITERPENE LACTONE HISTAMINE LIBERATOR FROM *THAPSIA GARGANICA*

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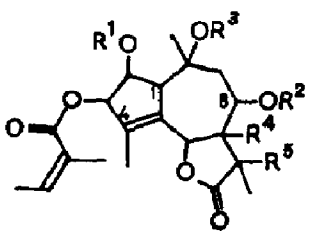
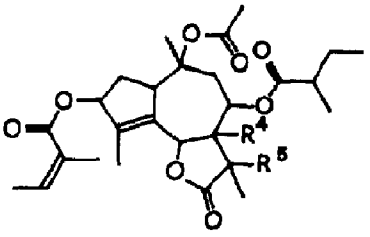
Summary: The major skin irritating constituent of the root of *Thapsia garganica* L. has been shown to be a hexaoxygenated-C_6-guaianolide esterificated with four carboxylic acids.

The skin irritating effects of the root of *Thapsia garganica* L. (Apiaceae = Umbelliferae) were described by Hippokrates about 400 B.C., and the root has been used in folk medicine ever since. Pharmacological investigations of the active principles have revealed some very interesting histamine liberating effects of these compounds.[1,2] We now report the structure elucidation of the major skin irritant (named thapsigargin, **1a**) present in the root.



(1 a-f)



(2 a,b)

a R^1=$OC(CH_2)_6CH_3$; R^2=$OC(CH_2)_2CH_3$; R^3=$OCCH_3$; R^4=R^5=OH
b R^1=$OC(CH_2)_6CH_3$; R^2=$OC(CH_2)_2CH_3$; R^3=$OCCH_3$; R^4,R^5=-O-
c R^1=$OC(CH_2)_6CH_3$; R^2=H; R^3=$OCCH_3$; R^4,R^5=-O-
d R^1=H; R^2=$OC(CH_2)_2CH_3$; R^3=H; R^4,R^5=-O-
e R^1=R^2=H; R^3=$OCCH_3$; R^4,R^5=-O-
f R^1=R^2=R^3=H; R^4,R^5=-O-

The compound, isolated as previously described,[1] was obtained as a colourless amorphous powder. The i.r. spectrum (CCl_4) showed a carbonyl band at 1780 (γ-lactone) and a number of carbonyl bands between 1750-1700 cm^{-1} (esters). The identity of the acyl residues was determined by transesterifications into methyl esters by treatment with 0.1 M sodium methanolate. Combined GC-MS investigations of the methyl esters formed using authentic samples as references showed thapsigargin to give rise to methyl esters of acetic, butyric, angelic, and octanoic acid.

FD-MS produced a reproducible peak at m/e 651 (**1a**, M+1). A molecular ion could not be detected in the EI-mass spectrum but a prominent peak was found at m/e 446.1944 ($C_{24}H_{30}O_8$). If this signal originates by elimination of octanoic and acetic acid, the molecular formula of **1a** is $C_{34}H_{50}O_{12}$. Thus the molecule is assumed to be a sesquiterpene lactone bearing six hydroxyl functions,

four of which are esterificated.

Apart from the characteristic signals due to the four acyl residues the 270 MHz ^{1}H n.m.r. spectrum ($CDCl_3$) displayed methine protons resonances at δ 5.68 (H_2,H_6,H_8), 5.49 (H_3) and 4.39 (H_1). Signals at δ 1.39, 1.45 and 1.84 were assigned to three methyl groups. The ^{1}H n.m.r. spectrum also revealed the signal due to one of the H_9 protons as the A part of an ABX system at δ 3.10 (J_{gem}14 Hz, J_{vic} small). The B part was hidden under the signals originating from the α protons of octanoic and butyric acid (δ 2.3). These n.m.r. data resemble closely those of trilobolide (**2a**).[3] Also ^{13}C n.m.r. spectroscopical investigations confirmed the skeleton of thapsigargin to resemble that of **2a**. Thus thapsigargin is concluded to be a 3,7,8,10,11-oxygenated-$\Delta^{4(5)}$-C_6-guaianolide further substituted with one hydroxyl function. In the spectrum of **2a** a very characteristic signal due to one of the H_2 protons is observed at δ 2.5. The absence of this signal in the spectrum of **1a** located the additional oxygen substituent to the two position.

The vicinal location of the two free hydroxyl groups was evident from the formation of an epoxide (**1b**, m.p. 110-111°C, $[\alpha]_D^{20}$ -11°, (c 0.09, MeOH)) by treatment of **1a** with a solution of thionyl chloride in pyridine. As was the case for **1a** and **2a** analogous protons in the spectra of **1b** and **2b** were found to have similar chemical shift values.

The locations of the four carboxylic acid residues are based on ^{1}H n.m.r. spectroscopical investigations of four of the products (**1c-f**) formed by partial saponification of **1b** using 0.3 M methanolic potassium hydroxide. The chemical shift of H_2 changed from δ 5.68 to 4.22 when the octanoyl group was lost. Analogously the δ value of H_8 was shifted from 5.68 to 3.80 when the butyryl group was lost. No similar change of the signal due to H_3 is observed in the spectra of **1e** and **1f**.

Thapsigargin is an example of a pharmacological highly active sesquiterpene not containing an α,β-unsaturated carbonyl moiety on the sesquiterpene nucleus. The biological activity of a number of other sesquiterpene lactones has been explained by the presence of that moiety.[4,5]

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