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NEW DITERPENOID IRRITANTS FROM EUPHORBIA INGENS

H. J. Opferkuch and E. Hecker[+)]

Institut für Biochemie, Deutsches Krebsforschungszentrum,

Heidelberg, Germany

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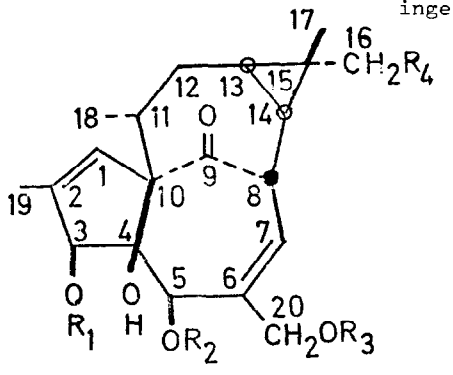
Apart from the well known biologically inactive triterpenes Euphol and Euphorbol (1, 2), the latex of Euphorbia ingens contains esters of a variety of diterpene alcohols. Some of these are responsible for the irritant activity and possibly also for the cocarcinogenic activity of the latex (3, 4). By appropriate combinations of chromatography and multiplicative distribution methods (5) with biological tests for irritant activity separation of the acetone extract of the latex was accomplished and the highly irritant Euphorbia factors I_1, I_5, I_6 and the non-irritant compounds I_2 and I_4 were isolated. As yet Euphorbia factor I_1 was recognized as the 3-hexadecanoate (6,7), and compound I_2 as the 20-hexadecanoate (7, 8) of the tetracyclic diterpene ingenol (9). Compound I_4 was identified as the 3,7,12-triacetate-8-nicotinate of the new macrocyclic diterpene ingol (10).



ingenol[++)]: R_1, R_2, R_3, R_4 = H

I_1 : R_1 = hexadecanoate, R_2,R_3,R_4=H

I_2 : R_1,R_2,R_4=H,R_3 = hexadecanoate

I_6 : R_1 = 2,4,6-decatrienoate,R_2,R_3,R_4=H

1 : R_1,R_2,R_3 = H, R_4 = OH

2 : R_1,R_2,R_3 = $COCH_3$, R_4 = $OCOCH_3$

I_5 : R_1 = 2,4,6-decatrienoate, R_2,R_3 = H,

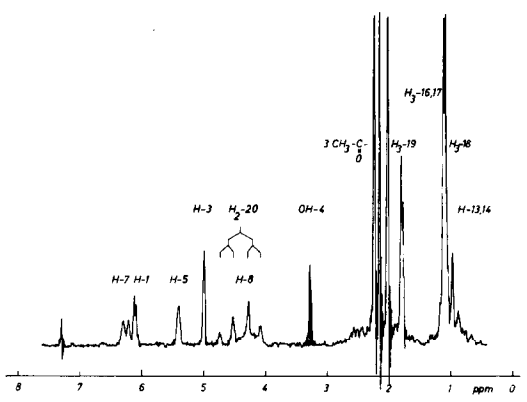
R_4 = $OCOC_4H_7$ (angelic acid)

+) Dedicated to Prof.Dr.H.Bredereck, member of the Kuratorium of the German Cancer Research Center on occasion of his 70th birthday.

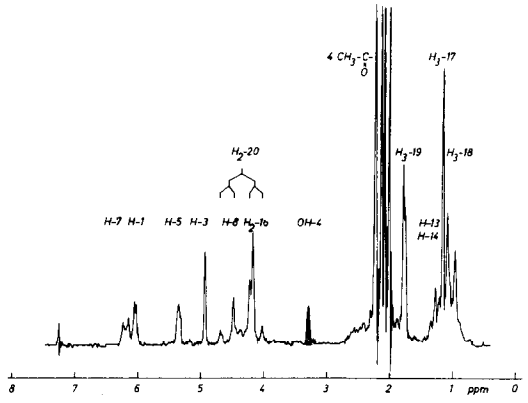
++) For the rules governing the signs to indicate stereochemistry, see (11).

The hitherto unknown Euphorbia factor I_6 is a resinous monoester of ingenol [mw : 496 ; ir (CH_2Cl_2): 3670 , 3570 , 3510 , 3420 , 1715 , 1630 , 1610 cm^{-1}; uv (CH_3OH): λ_{max}: 192 , 210 , 302.5 nm (18 000 , 14 000 , 27 700); nmr (δ, $CDCl_3$): 7.85 (1 H), 6.8 - 5.7 (7 H), H-3: 5.63, H-8: 4.3, H_2-20:4.13, H-5: 4.04 , H_3-19: 1.8 , H_3-16, H_3-17: 1.08 , 1.05 ppm]. The parent alcohol is identified by hydrolysis of I_6 and acetylation of the reaction product to ingenol-3,5,20-triacetate (9) with Ac_2O/Py. According to the spectral data of I_6, OH-3 of ingenol is esterified with 2,4,6-decatrienoic acid. The stereochemistry of the double bonds in this acid remains to be established.

Besides ingenol (9) and ingol-12-acetate (10) base catalysed transesterification of the unresolved fraction of diterpene esters yields a third and hitherto unknown diterpene alcohol 1 : 16-hydroxy-ingenol (mw: 364). By acetylation of 1 with Ac_2O/Py the tetraacetate 2 $C_{28}H_{36}O_{10}$ is obtained [mw: 532.2339 ; ir (KBr): 3450 , 1735 , 1640 cm^{-1}; uv (CH_3OH): λ_{max}: 193, 285 nm (15 3000 , 260); nmr (δ, $CDCl_3$, see also chart 1b): H-7: 6.25 , H-1: 6.01 , H-5: 5.4 , H-3: 5.0 , H_2-20: 4.4 $\pm$ 0.2 ; J_{AB}: 13 Hz , H_2-16: 4.22 , H-8: 4.2 , CH_3CO: 2.27 , 2.12 , 2.08 , 2.0 , H_3-19: 1.77 , H-13 , H-14: 1.4 - 0.9 , H_3-17: 1.15 , H_3-18: 1.0 , OH-4 (exchangeable): 3.36 ppm]. The downfield shifts of the signals for H-13 and H-14 in 2 with respect to the corresponding signals in ingenol-3,5,20-triacetate (9) indicate that the additional hydroxyl group is at the 16 position of ingenol. Thus the structure of 2 is analogous to that of 16-hydroxy-phorbol (12). In comparison with analogous signals in ingenol-3,5,20-triacetate (9) the additional OAc-group in 2 does not influence the chemical shifts of OH-4ß, H-8ß, H-11ß, and H-12ß as would be expected for an acetate at the 16 position rather than at the alternative 17 position. The circular dichroism of 2 $\Delta\varepsilon$: 278 (-0.12) , 300 (+0.64) , 311 nm (+0.715) is in agreement with the data of ingenol-3,5,20-triacetate (9).



1a



1b

Chart 1: 60 MHz nmr spectra of
a: ingenol-3,5,20-triacetate and
b: 16-hydroxy-ingenol-3,5,16,20-tetraacetate (2) in $CDCl_3$ with TMS (δ =0.00 ppm) as internal standard.

Euphorbia factor I_5 is an ester of 16-hydroxy-ingenol of the molecular formula $C_{35}H_{46}O_8$ (mw: 594.3223, peak matching). Its fragmentation indicates that the acid residues are $C_9H_{13}COOH$ and C_4H_7COOH, the latter being esterified with a primary hydroxyl group. Further spectral data are as follows: ir (KBr): 3450, 1715, 1640, 1615 cm^{-1}; uv (CH_3OH):λ_{max}: 208 , 301.5 nm (29 000 , 30 600); nmr (δ, $CDCl_3$): 7.78 (1 H) , 7.0 - 5.7 (8 H) , H-3: 5.63 , H_2-16 , H-8: 4.3 , H_2-20: 4.1 , H-5: 4.04 , 2 - CH_3: 2.05 , 1.92 , H_3-19: 1.8 , H_3-17: 1.16 , H_3-18: 0.98 , 3-OH (exchangeable): 4.65 , 3.75 , and 2.94 ppm. These spectral data and their comparison with the data of I_6 suggest that in I_5 OH-3 of 16-hydroxy-ingenol is esterified with 2,4,6-decatrienoic acid and OH-16 of 16-hydroxy-ingenol with angelic acid. The stereochemistry of the double bonds of 2,4,6-decatrienoic acid in I_5 appears to be identical with that in I_6.

On the mouse ear (5) compound I_6 shows an irritant dose 50 (ID_{50}) of 0.02 µg/ear and I_5 an ID_{50} of 0.004 µg/ear. Thus the irritant activity of I_5 is about one half of that of croton oil factor A_1 (5) and I_6 is about twice as active as A_1.

Acknowledgement

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