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INGOL - A NEW MACROCYCLIC DITERPENE ALCOHOL FROM EUPHORBIA INGENS

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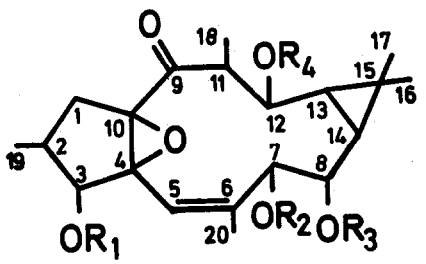
The acetone soluble compounds of the latex of Euphorbia ingens[++)] can be separated by combination of adsorption chromatography and Craig-distribution (1). Besides irritant and tumor promoting derivatives of the diterpene INGENOL (2-4) an additional compound 1 has been isolated. Compound 1 is the 3,7,12-triacetate-8-nicotinate of a new, polyfunctional diterpene INGOL[+++)] 2 (5).



1: R_1=R_2=R_4= acetate; R_3= nicotinate
2: R_1=R_2=R_3=R_4= H
3: R_1=R_2=R_3= H; R_4= acetate
4: R_1=R_2=R_3=R_4= acetate
5: R_1=R_2=R_3= benzoate; R_4= acetate
18: R_1=R_2= H; R_3= tigliate; R_4= acetate
19: R_1=R_2=R_4= acetate; R_3= tigliate

For compound 1 high resolution mass spectrometry yields the molecular formula $C_{32}H_{39}NO_{10}$ (597.2575 , calc. 597.2572). Its nmr spectrum (C_6D_6) shows the presence of three acetates (δ : 1.69 , 1.78 , 1.82 ppm, 3x3H, singlets) and one nicotinate (δ : 9.36 : H-2'; 8.34 : H-6'; 7.95 : H-4'; 6.5 ppm : H-5').

Base catalysed transesterification of 1 yields the monoacetate 3, $C_{22}H_{32}O_7$ [mw: 408 , nmr ($CDCl_3$): H-12: 4.85 ppm, $J_{12,13}$= 10.5 Hz, $J_{12,11}$= 4 Hz] , which on acetylation with Ac_2O/Py provides the tetraacetate 4 (mw: 534 , uv: λ_{max}: 198 , 227 , 297 nm, ε = 13 500, 2 920,44). Treatment of 3 with C_6H_5COCl/Py yields the acetate-tribenzoate 5 (mw: 720). From the nmr spectrum of 4 and decoupling experiments ($CDCl_3$, C_6D_6) the structural elements A to D may be deduced.

+)Dedicated to Prof.Dr.Th.Wieland, member of the Kuratorium of the German Cancer Research Center, on occasion of his 60th birthday.

++) We are greatly indebted to Dr.R.Dyer, Pretoria, for supply of latex

+++) The structure of 1 was presented by H.J.O. at the Colloquium Spektroscopicum Internationale XVI, Heidelberg, on October 5, 1971 (5).

Reduction of 4 with $NaBH_4$ furnishes the corresponding di-hydro compound 6 [mw: 536, ir(CH_2Cl_2): 3 605 cm^{-1}, uv: λ_{max}: 204 nm, ε = 10 900, nmr($CDCl_3$): H-9: 3.94 ppm (d), $J_{9,11}$= 8Hz]. Methanolysis of 6 with sodium methoxide in methanol yields the pentol 7, with no carbonyl absorption band in the ir spectrum indicating presence of only one carbonyl function in INGOL. The ^{13}C nmr spectra (noise decoupled and off resonance) of the tetraacetate 4 suggest that it contains either a ditertiary ether or a ditertiary epoxide group. $LiAlH_4$ reduction of 4 yields a hexol 8 which forms on acetylation (Ac_2O/Py) a pentaacetate 9 with one free tertiary hydroxyl group [mw: 580, ir (CH_2Cl_2): 3670 cm^{-1}, uv: λ_{max}: 194 nm, ε = 10 600] . These findings are compatible with existence of a ditertiary epoxide group in 2.

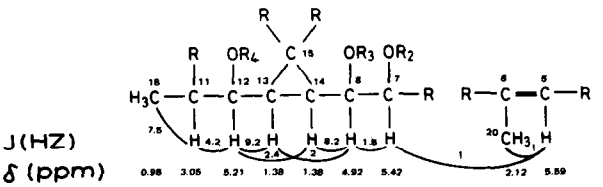
Oxidation of 4 with OsO_4 yields the diol 10 [mw: 568 , ir(CH_2Cl_2): 3595 , 3 450 cm^{-1}, nmr($CDCl_3$): H-5: 4.31 ; H_3-20: 1.3 ppm] whereas treatment with 3-Cl-perbenzoic acid leads to the epoxide 11 [mw: 550 , nmr($CDCl_3$): H-5: 3.27; H_3-20: 1.72 ppm] . These reactions confirm the presence of one double bond.

Under strong alkaline conditions (1 molar KOH/CH_3OH) 3 loses acetic acid to yield compound 12, containing a cyclopropyl-vinyl-ketone function [mw: 348.1930 , calc. 348.1937 for $C_{20}H_{28}O_5$, ir(KBr): 1 685 , 1 610 cm^{-1}, uv: λ_{max}: 195 , 279 nm, ε = 15 350 , 8 100, nmr ($CDCl_3$): H-12: 5.68 ; H_3-18:1.91ppm].

$NaIO_4$ oxidation of 7 yields 13 with an α,β-unsaturated aldehyde group [mw:: 366 , ir(KBr): 2 725 , 1 690 , 1 645 cm^{-1}, uv: λ_{max}: 192 , 244 , 310 nm, ε = 3 950 , 9800, 105 , nmr(d_5-pyridine): H-7: 9.54 ppm] . The second aldehyde function to be expected at position 8 forms a semi-acetale group, most probably with OH-12. The above results allow combination of the partial structures A and B to give E.

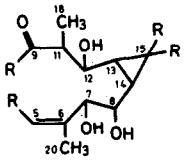
From the products of transesterification of 9 with sodium methoxide in methanol, the diacetate 14 may be isolated [nmr(d_5-pyridine): 2.25 , 2.18 : 2x3H; H-9: 4.30 ppm (d,$J_{9,10}$= 7 Hz)]. H-9 appears in the nmr spectrum as a doublet, coupling only with H-10. This result suggests that in 8 the α-epoxy-ketone group is opened by $LiAlH_4$ to yield as expected (7) a 4,9-dihydroxy group.

The acetonide 15 [mw: 448 , nmr($CDCl_3$): 1.50 , 1.37 ppm, 2x3H, s] may be obtained from 3 by treatment with acetone/p-CH_3-C_6H_4-SO_3H. Further information regarding the nature of the functional groups neighbouring the epoxide may be deduced from the spectra of compound 17, obtained from 15 by $LiAlH_4$ reduction (16: mw 410) and subsequent $NaIO_4$ oxidation of 16. 17 shows an α,β-unsaturated ketone group [ir (KBr): 3 420 , 1 687 , 1 632 cm^{-1}, uv: λ_{max}: 193 , 241 , 307.5 nm, ε = 4 800 , 5 800 , 230 , nmr($CDCl_3$): H-5: 6.30 : H_3-20: 1.94 ppm]. These data prove the connection of C-4 with C-3, C-5 and C-10. The interlinking of C-1 with C-10 and the substitution of the cyclopropane carbon C-15 with the methyl groups CH_3-17 (partialstructure D) follow unambigously from these findings. Thus INGOL 2 exhibits the same tricyclic carbon skeleton as LATHYROL

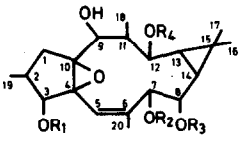


(C_6D_6) ; $R_1 = R_2 = R_3 = R_4 = COCH_3$, $R \neq H$

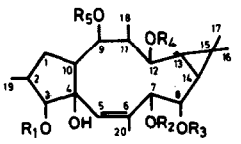
A B C D



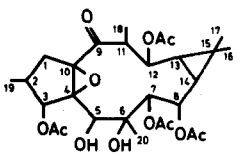
E



6 : $R_1=R_2=R_3=R_4=COCH_3$
7 : $R_1=R_2=R_3=R_4=H$



8 : $R_1=R_2=R_3=R_4=R_5=H$
9 : $R_1=R_2=R_3=R_4=R_5=COCH_3$
14 : $R_1=R_4=COCH_3$; $R_2=R_3=R_5=H$
16 : $R_1=R_4=R_5=H$; $R_2+R_3=>C(CH_3)_2$



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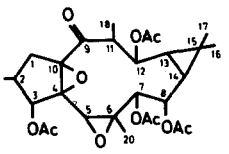

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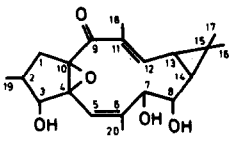

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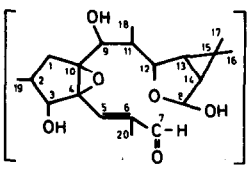

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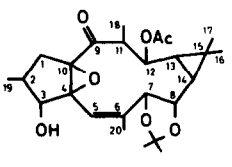

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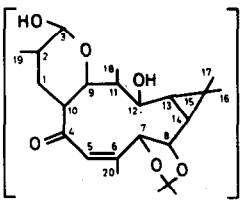
Li Al H_4 → 16 → $NaJO_4$



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and its derivatives (6) and as BERTHYADIONOL (8). Biogenetically it be formed from geranylgeranyl- pyrophosphate via CASBENE (9) as suggested already (6) for the macrocyclic diterpenes mentioned above.

For ingol 2 no stereochemical assignments are implied.

According to the following nmr data of 3, 4 and 5, compound 1 is the 3,12-triacetate-8-nicotinate of INGOL 2.

	H-3	H-7	H-8	H-12
3	4.36	4.21	3.47	4.85
4	5.37	5.09	4.59	4.89
5	5.42	5.56	4.96	5.02
1	5.38	5.23	4.90	4.90

In the mouse ear assay system (1) neither compound 1 nor any other derivative of ingol 2 shows irritant activity in doses up to 100 mμM/ear.

From one of the non-irritant fractions isolated from the latex a chromatographically uniform mixture of further tetraesters of 2 is obtained. This mixture is not amenable to separation by chromatographic methods, but mass spectrometric analysis shows the presence of substances with the following molecular formulae e.g.: 596.2627 = $C_{33}H_{40}O_{10}$; 576.2941 = $C_{31}H_{44}O_{10}$; 574.2774 = $C_{31}H_{42}O_{10}$. Base catalysed transesterification of this fraction yields besides 3 and other partially esterified ingol derivatives the 12-acetate-8-tigliate (18) of 2 [mw: 490 , uv: λ_{max}: 207.5 nm, ε = 17 900 ; nmr($CDCl_3$): tigliate residue: 6.94 , 1.87 (3H), 1.81 (3H,d,J= 6 Hz); H-5: 5.9 ; H-12: 4.88 ; H-8: 4.38 ; H-3: 4.33 ; H-7: 4.28 ppm]. Acetylation of 18 with Ac_2O/Py provides the 3,7,12-triacetate-8-tigliate (19) of 2, (mw: 574) which is one of the constituents of the above mentioned mixture of tetraesters.

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