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RESINIFERATOXIN AND OTHER ESTERS OF NOVEL POLYFUNCTIONAL DITERPENES FROM EUPHORBIA RESINIFERA AND UNISPINA

M. Hergenhahn, W. Adolf and E. Hecker[+]

Institut für Biochemie, Deutsches Krebsforschungszentrum, Heidelberg, Germany

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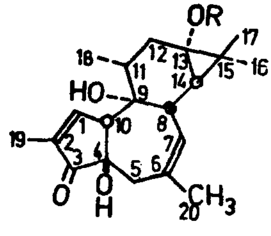
From a dried methanolic preparation of fresh latex of E. resinifera Berg. two yellow-orange staining (vanillin/sulfuric acid (V/S), 110°C, 1 min.[1]), non irritant compounds 1 and 2 as well as three blackish-brown staining irritant Euphorbia factors RL20 (3), RL9 (5) and RL14 (6) were isolated. Similarly, an Euphorbia factor U_1, identical with 5 (MS, PMR, IR and UV) was isolated from an acetone extract of latex of E. unispina N.E.Br. Factors RL9 and U_1, respectively, exhibit extremely high irritant activity on the mouse ear 2-4 h after application.

1: M^+402, R_f 0.65 (ether: hexane 5:1 on silica gel) 0.096% (by weight of dried latex). 2: M^+ 414, R_f 0.65 (ether:hexane 5:1), 0.02%. The PMR and mass spectra of both 1 and 2 indicate the presence of the same diterpene alcohol $C_{20}H_{28}O_4$ and reveal a general similarity to those of 12-deoxy-phorbol-13-isobutyrate[2-4] and 12-deoxy-phorbol-13-angelate[3,4], respectively. However, a primary vinylic hydroxymethylene function is absent whereas an additional vinylic methyl group (H_2-20) is present in 1 and 2. Hydrogenolytic deacetylation[1] of 12-deoxy-phorbol-13-isobutyrate-20-acetate yields 1. Consequently 1 is 12,20-dideoxy-phorbol-13-isobutyrate and 2 12,20-dideoxy-phorbol-13-angelate (for the rules governing the signs indicating the configuration of asymmetric centers see[1]).



1: $R=COCH(CH_3)CH_3$

2: $R=COC(CH_3)=CHCH_3$

3: (M^+-18) 628; R_f 0.45 (ether, on silica gel); 0.04% of dried latex. PMR: δ_{TMS}, $CDCl_3$, 7.47, m, H-1; 5.53, m, H-7, partially superimposed on H-14; 5.47, d, H-14, partially superimposed on H-7; 5.02, AB, H_2-17; 4.4, broad s, H_2-20; 3.7, m, H-8, superimposed on signal of an aromatic methoxy group; 3.04, m, H-10; 2.32, 2.25, AB, H_2-5, 2.05, m, H-11; about 2.0, m, H_2-12, superimposed on H-11; 1.8, m, H_3-19, superimposed on H_3-16; 1.78, broad s, H_3-16, superimposed on H_3-19; 0.92;d,

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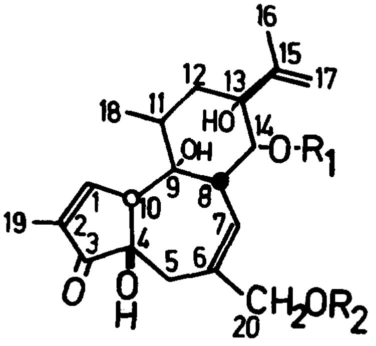
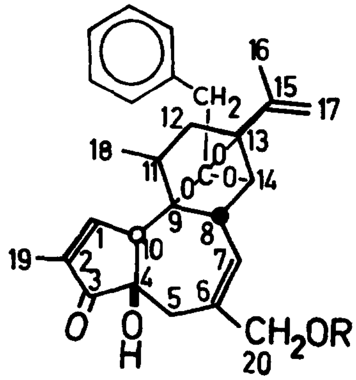
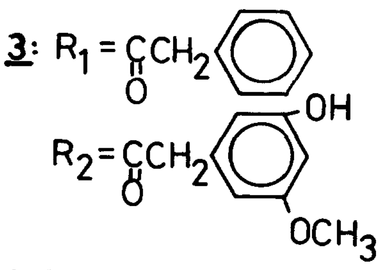
[+] Dedicated to Prof.Dr. E. Biekert, Director, KNOLL AG, Ludwigshafen/Rhein, on occasion of his 50th birthday.

J=6.5 cps, H_3-18; 3.2-2.2, 3 hydroxyl groups; 7.35, s, 5 protons; 3.51, s, 2 protons; 6.78, broad s, 3 protons; 3.86, s, aromatic methoxy group; 5.75, phenolic OH; 3.8, s, 2 protons.

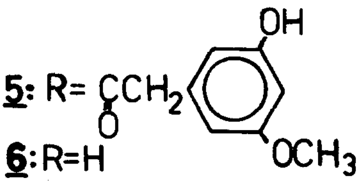
The PMR signals attributed to the parent alcohol of 3 reveal a general similarity to those of 12-deoxy-phorbol in its esters[2,4]. At variance with 12-deoxy-phorbol esters, the signals of a geminal dimethyl group are not present in the PMR spectrum of 3; instead the AB signal of two methylene protons at 5.02 ppm and the singlet of an olefinic methyl group at 1.8 ppm (overlapping the signal of H_3-19) may be attributed to an isopropenyl group. The signal of the proton at 5.47 ppm is characteristic for a proton geminal to an acyloxy group. By double resonance experiments this proton is shown to couple with H-8, and hence is H-14. This is in contrast to 12-deoxy-phorbol-esters in which H-14 is a cyclopropane proton. By deuterium exchange three tertiary hydroxyl groups (OH-4, OH-9, OH-13) and one phenolic hydroxyl group (OH-3') are shown to be present. The shapes and positions of the PMR signals of protons H-1, H_3-19, H_2-5, H-7, H_2-20, H-11, H_3-18, H_2-12 are very similar to those of 12-deoxy-phorbol-esters. Therefore, the structure and configuration 4 is proposed for the polyfunctional parent diterpene of Euphorbia factor RL20 which is called resiniferonol. The positions of the two acyl residues in 3 will be determined below.







4: $R_1=R_2=H$



3 yields a less polar compound either spontaneously, by warming to 35°C in CCl_4 or by treatment with traces of trifluoracetic acid. According to R_f and spectroscopical data it is identical with the extremely irritant Euphorbia factors RL9 and U_1 (5), respectively, as obtained from the latices of E. resinifera and E. unispina. 5: M^+ 628, R_f 0.41 (ether: hexane 5:1 on silica gel), 0.26 % of dried E. resinifera latex; E. unispina: 0.3 % of acetone extract of fresh latex; PMR: δ7.42, m, H-1; 5.84, m, H-7; 4.7, AB, H_2-17; 4.53, AB, H_2-20; 4.18, d,J=7 cps, H-14; 3.05, m, H-10, H-8; about 2.6, m, H-11; about 2.2-1.9, m, H_2-12; 2.23, 2.12, AB, H_2-5, superimposed on OH-4; 1.81, m, H_3-19; 1.52, broad s, H_3-16; 0.96, d,J=7.5, H_3-18; about 2.5-2.0, OH-4. The signals at 7.27, m, 5 protons; 3.2, s, 2 protons suggest the presence of a benzyl group. The signals at 6.76, AB, 3 protons; about 5.5, phenolic OH; 3.85, s, aromatic methoxy group, and 3,55, s, 2 protons may be ascribed to an additional substituted benzyl group. At variance with 3 the signal of H-14 is shifted from 5.47 to 4.18 ppm and the signal of one of the two benzylic methylene groups from 3.51 to 3.2 ppm. Other signals are but slightly shifted. As compared to 3 only two exchangeable signals (OH-4, OH-3') are left. The major product of $LiAlH_4$ reduction of 5 does not contain a carbonyl absorption at about 1730-1700 cm^{-1} while the NMR signals of one of the benzyl groups at 7.27 and 3.2 ppm are retained. This suggests the presence of a phenylacetic acid orthoester group. Its presence is further substantiated by the position of the PMR signals of the benzyl protons of the analogous methyl orthophenylacetate at 3.08 and 7.27 ppm, respectively. By mild acid-catalyzed transesterification[1] of 5 a product 6 can be obtained which is identical with the irritant Euphorbia factor RL14. According to its spectroscopical data it is devoid of the arom. substituted benzyl group, which, therefore, represents a substituted phenylacetyl residue at O-20.

For Euphorbia factors RL9 and U_1, respectively, the structure and configuration 5 is proposed. By analogy, for its precursor RL20 structure 3[5] and for its transesterification products 6 follow. Similar toxins, containing epoxy-hydroxy-derivatives of resiniferonol have been isolated from Hura crepitans and from Hippomane mancinella (huratoxin[6,7] and mancinellin[7];Hura, Hippomane; Euphorbiaceae) as well as from Daphne species (daphnetoxin[8], mecerein[9,10], Daphne; Thymelaeaceae) and from Lasiosiphon burchelli[11] (Gnidia; Thymelaeaceae).

RL9 (5) is the most irritant[4] diterpene ester hitherto isolated from Euphorbiaceae and Thymelaeaceae. It is called resiniferatoxin . RL20 (3) is less irritant than RL9 and may be considered a (biogenetic) precursor of the latter. Hence the name proresiniferatoxin is proposed for RL20.

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Acknowledgements

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