STRUCTURE–ACTIVITY RELATIONS OF POLYFUNCTIONAL DITERPENES OF THE DAPHNANE TYPE. I. REVISED STRUCTURE FOR RESINIFERATOXIN AND STRUCTURE–ACTIVITY RELATIONS OF RESINIFERONOL AND SOME OF ITS ESTERS

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Abstract.—Base-catalyzed transesterifications of resiniferatoxin afforded the 9,13,14-orthophenylacetate (III), as well as (4-hydroxy-3-methoxyphenyl)acetic acid methyl ester (II). Reesterification of III with homovanillic acid showed that the previously proposed structure for resiniferatoxin has to be revised to resiniferonol-9,13,14-orthophenylacetate-20-(4-hydroxy-3-methoxyphenyl)acetate (I). From III, different 20-esters (I, IV–IX) were prepared. The orthophenylacetate III was cleaved by acidic hydrolysis to yield the 14-phenylacetate X. Subsequent alkaline transesterifications of X afforded the parent alcohol resiniferonol (XI). Starting from XI, the 14,20-diesters XII and XV and the 9,13,14-orthoesters XIII, XIV, XVI, XVII and XVIII were obtained. All esters were tested for irritant activity on the mouse ear. Some aspects of structure-activity relations of irritancy of resiniferonol esters are established and discussed.

Resiniferatoxin (**I**), isolated from the irritant latices of the plant species *Euphorbia resinifera* (1), *Euphorbia unispina* (1) and *Euphorbia poisonii* (2) represents an *Euphorbia* factor extremely irritant to mucous membranes of the human nose, lips and throat.   This is reflected also in the test for irritant activity on the mouse ear (3) where **I** exhibits the lowest irritant dose 50 (ID_{50}) of all irritant factors so far isolated from Euphorbiaceae and Thymelaeaceae (for reviews see 4, 5).   If the results are read 3–5 hours after application of **I** to the ear of NMRI-mice, an ID_{50} of about 1/1000 of that of Croton oil factor A_1 [TPA, 12-O-tetra-decanoylphorbol-13-acetate (6) was found.   Yet, 24 hours after application, when the diterpene esters tested so far exhibit their maximal activity (7) the ear redness caused by **I** has already disappeared.   These unusual findings provoked in-depth studies of structure-activity relations of **I** and related polyfunctional diterpenes of the daphnane type in our laboratory.

A report (8) on some aspects of structure-activity relations of resiniferonol esters prompts us to present (i) conclusive evidence that the structure of **I** proposed previously (1) has to be revised and (ii) some results of chemical modification of **I** to evaluate their influence on the ID_{50} in NMRI-mice.   Chemical modifications were accomplished by transesterification of the C–20 acid moiety in **I** and re-esterification with various acids and also by cleavage of the orthoester acid moiety in **I** and formation of orthoesters with acetic and tetradecanoic acid.

EXPERIMENTAL

Biological assay.—Irritant doses 50 (ID_{50}) were determined on the mouse ear according to the standard procedure (3,6). The ear redness as developed 5 and 24 hours after application of the compound was evaluated. In all cases the maxima of the redness appeared around 5 hours or 24 hours after application. The results are expressed as ID_{50}^{hours} in nmoles/ear. All data on irritant activity are collected in table 1.

Spectra.—Mass-spectra (ms) were measured with a Varian MAT 711 spectrometer, uv-spectra (uv) with a Beckman DK 2a far uv spectrometer, ir-spectra (ir) with a Perkin Elmer spectral photometer 521 and [1]H-nmr-spectra (nmr) with a Bruker HX 90 spectrometer. Chemical shifts refer to tetramethylsilane ($\delta = 0.00$ ppm) as internal standard and deutero-chloroform as solvent unless otherwise indicated.

Chromatographic methods.—For column chromatography, Merck silica gel 0.05–0.20 mm was used, deactivated with 13% water. For preparative thin-layer chromatography (prep. tlc), either precoated plates (0.5 mm, Merck) or Merck silica gel PF_{254} were used and for analytical tlc, precoated plates (0.25 mm, Merck) were used. Spots were detected under

uv-light (254 nm) and/or by spraying with vanillin/sulfuric acid followed by heating up to 110° for 2–5 min. Unless otherwise stated, R_f values refer to the solvent system used for purification of compounds by prep. tlc. A Packard gas chromatograph, model 420, was used for gas-chromatographic analysis (glc) of acid methyl esters.

SYNTHETIC REACTIONS.—Work-up (*procedure A*): Excess phosphate buffer (pH appr. 7) was added and stirred for a time if necessary. Subsequently the mixture was extracted three times with ethyl acetate. The organic layer was dried with sodium sulphate and finally removed in a rotary evaporator. Work-up (*procedure B*): the same as procedure A except that the ethyl acetate was additionally extracted with dilute acid (excess of approx. 1 M HCl) and thereafter a second time with phosphate buffer.

ISOLATION PROCEDURE FOR RESINIFERATOXIN (I).—A dried latex preparation (650 g) of *Euphorbia unispina* L. was shaken several times with 2 liters of acetone for 24 hours. After filtration of the insoluble residue and evaporation of the solvent, 120 g of an acetone extract was obtained. O'Keeffe distribution of the acetone extract in petroleum ether-methanol-water (15:10:0.5) (v=300/300 ml; z=11 elements) afforded 24 g of a hydrophilic fraction, which was filtered through a small silica gel column with ethyl acetate to yield 17.0 g of resinous material after evaporation of the solvent. This material was chromatographed on a silica gel column (3 kg of silica gel) with ether-petroleum ether (2:3). Subsequently, a fraction (570 mg) was obtained which was further purified by prep. tlc with petroleum ether-ether-acetone (1:1:1) as the solvent; 420 mg of tlc pure resiniferatoxin (I) was obtained; R_f value [ethyl acetate-petroleum ether (1:1)]: 0.37. For spectral data of I see (1).

BASE–CATALYZED TRANSESTERIFICATIONS OF I PROVIDING III.

a) I (400 mg) was treated with 80 ml of a 0.1 M solution of sodium methoxide in methanol for 2 hours. Work-up (procedure A) and prep. tlc with ethyl acetate-petroleum ether (2:1) yielded **226** mg of III R_f: 0.26; ms: parent ion m/e 464, fragment ions m/e 446, 428, 328, 310; nmr: 1-H: 7.42 (m), 5 aromatic H: 7.25 (m), 7-H: 5.85 (m), 16-H_2: 4.70 (s), 14-H: 4.23 (d, $J=2.5$ cps), 20-H_2: 4.09 (s), CH_2 (phenylacetate): 3.19 (s), 8-H, 10-H: 3.1 (m), 19-H_3: 1.80 (m), 17-H_3: 1.51 (s), 18-H_3: 0.95 ppm (d, $J=7$ cps); ir (CH_2Cl_2): 3600, 3555, 3450 (OH), 1700 (CO), 1640, 1625, 1600 cm^{-1} (C=C); uv (methanol): λ max (ϵ max): 320 (190), 234 (6550), λ (ϵ): 198 nm (20700).

b) I (30 mg) was treated with 3 ml of 0.1 M sodium methoxide in methanol for 1.5 hrs. Working up (procedure A) and prep. tlc with ether-petroleum ether (4:1) yielded 5 mg of II (R_f: 0.45) and 14 mg of III (R_f: 0.1). Spectral data of II: ms: parent ion m/e 196; nmr: as an authentic sample of II (synthesized from homovanillic acid); glc: column: 10% GESE 30 on chromosorb W/HP, carrier gas N_2, flow rate 25 ml/min., detector: FID, flow rates: H_2: 25 ml/min., synthetic air: 300 ml/min., retention time: as an authentical sample of II.

ESTERIFICATION OF III WITH (4–HYDROXY-3-METHOXYPHENYL) ACETIC ACID (HOMOVANILLIC ACID), PROVIDING I.—III (13 mg) was dissolved in 0.26 ml of dichloromethane including 5.4 mg of triethylamine. To this N-methyl-2-fluoropyridinium tosylate (16 mg) (9) was added. After 0.5 hrs the solvent was removed with a rotary evaporator and 0.26 ml of benzene-acetone (1:1) including 8.5 mg of triethylamine were added followed by 16 mg of solid homovanillic acid. The reaction mixture was stirred, heated to 60° for 1.5 hrs and finally worked-up (procedure B). Prep. tlc with ether-petroleum ether (4:1) yielded 7 mg of I. The spectroscopic data follows: ms: parent ion: m/e 628. Nmr ($CDCl_3+D_2O$): 1-H: 7.45 (m), 5 aromatic H: 7.30 (m), 3 aromatic H: 6.83 (s), 7-H: 5.88 (m), 16-H_2: 4.72 (s, br.), 20-H_2: 4.58 (s, br.), 14-H: 4.21 (d, $J=2.5$ cps), OCH_3: 3.89 (s), CH_2: 3.56 (s), CH_2: 3.22 (s), 8-H, 10-H: 3.08 (m), 19-H_3: 1.83 (m), 17-H_3: 1.52 (s, br.), 18-H_3: 0.96 ppm (d, $J=7$ cps); ir (CH_2Cl_2): 3540 (OH), 1728, 1703 (CO), 1640, 1625, 1605 cm^{-1} (C=C); uv (methanol): λ max (ϵ max): 281 (3660), 231 (13560), λ (ϵ): 198 nm (71200). All spectral data are in good agreement with those published for Euphorbia factors RL–9 and U_1 [resiniferatoxin, (I)] (1).

METHYLATION OF I, PROVIDING IV.—I (12 mg) was treated with 5 ml of a solution of diazomethane in diethylether for 30 min. After evaporation of the solvent and prep. tlc with chloroform-ethyl acetate (8:1) as the solvent product IV (3 mg) with higher R_f value than the starting material was recovered. It gave ms: parent ion m/e 642; nmr: signals at variance with those in the spectrum of I:2 singlets at 3.85 and 3.87 ppm corresponding to two OCH_3 groups.

ESTERIFICATION OF III WITH (3,4–DIMETHOXYPHENYL) ACETIC ACID (HOMOVERATRIC ACID), PROVIDING IV.—III (20 mg) was treated as described for the preparation of I from III except that (3,4-dimethoxyphenyl) acetic acid was added. Prep. tlc with ether-petroleum ether (4:1) as solvent afforded 10 mg of IV (R_f: 0.36). The ms and nmr spectra were identical with those of the methylation product of I (above).

ESTERIFICATION OF III WITH (3,5–DIMETHOXYPHENYL) ACETIC ACID, PROVIDING V.—Toluene (0.6 ml) including 39 mg of triethylamine and 20 mg of (3,5-dimethoxyphenyl) acetic acid were added to 13 mg of III. The acid was prepared from (3,5-dihydroxyphenyl) acetic acid (10) by standard methods for methylation (diazomethane and subsequent alkaline treatment (MeOH/H_2O/NaOH). The reaction mixture was heated to 90° for 1.75 hrs and, after work-up (procedure B), prep. tlc with ether-petroleum ether (2:1) (developed twice) afforded 6.5 mg of V. It gave ms: parent ion m/e 642; nmr: signals corresponding to the aromatic acid moieties: 5 aromatic H: 7.30 (m), 3 aromatic H: 6.42 (m), 2 OCH_3: 3.81 (s), 2 CH_2: 3.59 (s) and 3.22 ppm (s), all other signals are identical with those in the spectrum of I.

ESTERIFICATION OF **III**, PROVIDING **VI, VII, VIII, IX.**—These acylations were performed by standard procedures, i.e., 0.05 ml pyridine/toluene = 1/1 per mg of **III**, 6-fold molar excess of acid chloride or acetic acid anhydride, overnight stirring, work up: procedure B and purifications by prep. tlc.

RESINIFERONOL-9,13,14-ORTHOPHENYLACETATE-20-PHENYLACETATE   (**VI**).—Compound **VI** gave R_f [ether-petroleum ether (2:1)]: 0.31; ms: parent ion m/e 582; nmr: signals corresponding to the aromatic acid moieties: 10 aromatic H: 7.25 (m), CH_2: 3.64 (s) and 3.20 ppm (s), all other signals are identical with those in the spectrum of **I**.

RESINIFERONOL-9,13,14-ORTHOPHENYLACETATE-20-BENZOATE   (**VII**).—Compound **VII** gave R_f [ether-petroleum ether (1:1)]: 0.33; ms: parent ion m/e 568; nmr: signals corresponding to the aromatic acid moieties: 2H: 8.0–8,15, 3-H: 7.4–7.6, 5-H: 7.3 (m), CH_2: 3.24 ppm (s), all other signals correspond to those in the spectrum of **I**.

RESINIFERONOL-9,13,14-ORTHOPHENYLACETATE-20-HEXANOATE   (**VIII**).—Compound **VIII** gave R_f [ether-petroleum ether (1:1)]: 0.26; ms: parent ion m/e 562; nmr: signals corresponding to the acid moieties: 5 aromatic H: 7.3 (m), CH_2: 3.22 (s), CH_2–CO: 2.25 (t, $J=7$ cps), $(CH_2)_3$: 1.26 (s), terminal CH_3: 0.9 ppm (pert. t).

RESINIFERONOL-9,13,14-ORTHOPHENYLACETATE-20-ACETATE   (**IX**).—Compound **IX** gave R_f [ether-petroleum ether (1:1)]: 0.13; ms: parent ion m/e 506; nmr: signals corresponding to the acid moieties: 5 aromatic H: 7.3 (m), CH_2: 3.21 (s), acetate: 2.06 ppm.

ACID HYDROLYSIS OF **III**, PROVIDING **X.**—**III** (120 mg) was dissolved in 120 ml of methanol, and 24 ml of 1 M hydrochloric acid were added. The reaction mixture was kept at 80° for 2.5 hours. After work-up (procedure A), besides 45 mg of starting material, 43.5 mg of **X** were obtained after prep. tlc with ethyl acetate-petroleum ether (2:1) as solvent; R_f: 0.18; ms: fragment ions m/e 464 (M^+-H_2O), 446, 346, 328, 310; nmr: 1-H: 7.53 (m), 5 aromatic H: 7.38 (s), 14-H: 5.56 (d, $J=2$ cps), 7-H: 5.40 (d, br., $J=7$ cps), 16-H_2: 5.04 (s), 20-H_2: 3.93 (s), CH_2: 3.83 (s), 8-H: 3.7–3.8 (partially superimposed with 20-H_2), 10-H: 3.08 (m), 5-H_2: 2.40 (s, br.), 17-H_3, 19-H_3: 1.80 (superimposed), 18-H_3: 0.92 ppm (d, $J=7$ cps); ir (CH_2Cl_2): 3580, 3450 (OH), 1730, 1700 (CO), 1623, 1598 cm^{-1} (C=C); uv (methanol): λ max (ϵ max) 330 (210), 232 (5800); λ (ϵ) 196 nm (21800).

BASE-CATALYZED TRANSESTERIFICATION OF **X**, PROVIDING **XI.**—**X** (97 mg) was treated with 10 ml of a 10^{-2} M solution of sodium methoxide in methanol for 60 min. Addition of excess phosphate buffer and extraction of the aqueous phase with butanol yielded 38 mg of **XI** after prep. tlc with dichloromethane-methanol (10:1) as solvent; R_f value: 0.14; ms (electron impact): fragment ions m/e 346, 328, 310, 292; ms (field desorption): parent ion m/e 364; nmr (D_2O): 1-H: 7.56 (m), 7-H: 5.72 (d, br.), 16-H_2: 4.99 (s), 14-H: 4.02 (s, br.), 20-H_2: 3.90 (s), 8-H, 10-H: 3.05–3.2 (superimposed), 5-H_2: 2.33 (s, br.), 17-H_3, 19-H_3: 1.64 (superimposed), 18-H_3: 0.88 ppm (d, $J=6$ cps); ir (KBr): 3380 (OH), 1690 (CO), 1623 cm^{-1} (C=C); uv (methanol): λ max (ϵ max): 330 (90); 236 (4300); λ (ϵ) 192 nm (13000).

ACETYLATION OF **XI**, PROVIDING **XII.**—**XI** (20 mg) was treated with 1.5 ml of pyridine and 0.8 ml of acetic anhydride for 15 hours. After work-up (procedure B), prep. tlc with ethyl acetate-petroleum ether (2:1) as solvent afforded 19 mg of **XII**. It gave R_f value: 0.55; ms: parent ion m/e 448, fragment ions m/e 430, 412, 388, 370, 328, 310, 292, 282, 264; nmr: 1-H: 7.50 (m), 7-H, 14-H: 5.56 (superimposed), 16-H_2: 5.10 (s, br) 20-H_2: 4.45, 8-H: 3.77 (d, br), 10-H: 3.16 (m), 5-H_2: 2.4 (AB), 17-H_3, 19-H: 1.88 (s), 1.85 (m), 18-H_3: 1.02 (d, $J=6$ cps), 2 acetyl: 2.25 and 2.08 ppm; ir (CH_2Cl_2): 3580 (OH), 1730, 1703 (CO), 1628 cm^{-1} (C=C); uv (methanol): λ max (ϵ max): 326 (110), 238 (6200); λ (ϵ) 194 nm (23200).

ACIDIC TREATMENT OF **XII**, PROVIDING **XIII.**—**XII** (15 mg) was treated with 2.5 ml of a 0.5% solution of perchloric acid in methanol for 5 hours. After work-up (procedure A), prep. tlc with ethyl acetate-petroleum ether (1:1) as solvent yielded 8.8 mg of **XIII**; R_f 0.19; ms: parent ion m/e 388, fragment ions m/e 370, 352, 328, 310; nmr: 1-H: 7,52 (m), 7-H: 5.82 (s, br.), 16-H_2: 4.99 (s), 4.88 (s, br.), 14-H: 4.28 (d, $J=2.5$ cps), 20-H_2: 4.08 (s), 8-H, 10-H: 3.10 (superimposed), 17-H_3, 19-H_3: 1.82 (dd), 1.78 (s), 18-H_3: 1.15 (d, $J=7$ cps), orthoacetate: 1.67 ppm.

ESTERIFICATION OF **XIII** WITH (3,5-DIMETHOXYPHENYL) ACETIC ACID, PROVIDING **XIV.**—**XIII** (9 mg) was acylated under the reaction conditions described for the acylation of **III**. After prep. tlc with ether-petroleum ether (2:1) as solvent (developed twice), 3 mg of **XIV** were obtained; R_f: 0.5; ms: parent ion m/e 566, fragment ions m/e 506, 410, 328, 310, 196, 179, 178; nmr: 1-H: 7.50 (m), 3 aromatic H: 6.4 (m), 7-H: 5.85 (m), 16-H_2: 5.0 (s), 4.89 (s), 20-H_2: 4.56 (AB); 14-H: 4.24 (d, $J=2.5$ cps), 2 OCH_3: 3.82 (s), CH_2: 3.56 (s), 8-H, 10-H: 3.0–3.2 (superimposed), 17-H_3, 19-H_3: 1.84 (dd), 1.78 (s), orthoacetate 1.68, 18-H_3: 1.15 ppm (d, $J=6$ cps).

ESTERIFICATION OF **XI** WITH TETRADECANOIC ACID, PROVIDING **XV.**—**XI** (37 mg) was suspended in 28 ml of benzene and 3.7 ml of pyridine, and 0.52 ml of tetradecanoyl chloride were added. After 7 hours of stirring in a nitrogen atmosphere, another 0.52 ml of tetradecanoyl chloride were added, and the reaction mixture was stirred overnight. Work-up (procedure B) yielded 73 mg of **XV** after prep. tlc with petroleum ether-ethyl acetate (4:1) as solvent; R_f: 0.07; ms: parent ion m/e 784; fragment ions m/e 766, 748, 556, 538, 520, 448, 328, 323, 310; nmr: 1-H: 7.53 (m), 7-H, 14-H: 5.5–5.6 (superimposed), 16-H_2: 5.08 (s, br.), 20-H_2: 4.45 (s), 8-H: 3.77 (d, br.), 10-H: 3.17 (m), 17-H_3: 1.84 (s), 19-H_3: 1.79 (dd), 18-H_3: 0.99 ppm (d, $J=6$ cps), all signals were assigned by decoupling experiments; ir (CH_2Cl_2): 3680, 3570 (OH), 1735, 1705 (CO), 1628,

1600 cm^{-1} (C=C); uv (methanol): λ max (ε max): 328 (190), sh 250 (4700); 225 (6150); λ (ε) 193 nm (16350).

ACIDIC TREATMENT OF **XV**, PROVIDING **XVI**.—**XV** (57 mg) was treated with 42 ml of a 0.5% solution of perchloric acid in methanol for 2 hours. After work-up (procedure A), 20 mg of the raw material **XVI** were purified by prep. tlc with petroleum ether-ethyl acetate (4:1) as solvent; R$_f$: 0.27; ms: parent ion m/e 766, fragment ions m/e 748, 738, 538, 520, 510, 492, 328, 310, 292, 264; nmr: 1-H: 7.52 (m), 7-H: 5.87 (m), 16-H$_2$: 5.01 (s), 4.87 (s, br.), 20-H$_2$: 4.52 (s), 14-H: 4.27 (d, J=2.5 cps), 8-H, 10-H: 3.10 (superimposed), 19-H$_3$: 1.85 (dd), 17-H$_3$: 1.76 (s), 18-H$_3$: 1.14 ppm (d, J=7 cps); ir (CH$_2$Cl$_2$): 3680, 3550 (OH), 1728, 1705 (CO), 1628, 1600 cm^{-1} (C=C); uv (methanol): λ max (ε max): 332 (80), 233 (6060); λ (ε) 191 nm (22500).

BASE-CATALYZED TRANSESTERIFICATION OF **XVI**, PROVIDING **XVII**.—**XVI** (37 mg) was treated with 12.3 ml of a 0.1 M solution of sodium methoxide in methanol for 1 hour. After work-up (procedure A), prep. tlc with ethyl acetate-petroleum ether (1:1) as solvent yielded 11 mg of **XVII**; R$_f$: 0.24; ms: parent ion m/e 556 (C$_{34}$H$_{52}$O$_6$, peak matching), fragment ions m/e 538, 520, 328, 310, 282; nmr: 1-H: 7.50 (m), 7-H: 5.82 (m), 16-H$_2$: 5.0 (s), 4.87 (s, br.), 14-H: 4.28 (d, J=2.5 cps), 20-H$_2$: 4.06 (s), 8-H, 10-H: 3.10 (superimposed), 19-H$_3$: 1.85 (dd), 17-H$_3$: 1.76 (s), 18-H$_3$: 1.13 ppm (d, J=7 cps).

ESTERIFICATION OF **XVII** WITH (3,5-DIMETHOXYPHENYL) ACETIC ACID, PROVIDING **XVIII**.— Toluene (0.5 ml) including 15 mg of triethylamine and 7.8 mg of (3,5-dimethoxyphenyl) acetic acid were added to 5 mg of **XVII** (for preparation of the acid see above) and thereafter 26 mg N-methyl-2-bromopyridinium tosylate (9). After 1.5 hours of heating at 90° the mixture was worked-up (procedure B). Prep. tlc with ether-petroleum ether (developed twice) yielded 1 mg **XVIII**; R$_f$: 0.40; ms: parent ion m/e 734 (C$_{37}$H$_{66}$O$_{14}$, peak matching), fragment ions m/e 716, 538, 520, 506, 328, 310, 282, 264; nmr: 1-H: 7.5 (m), 3 aromatic H: 6.42 (m), 7-H: 5.85 (m), 16-H$_2$: 5.01 (s), 4.88 (s, br.), 20-H$_2$: 4.55 (AB), 14-H: 4.24 (d, J=2.5 cps), 2 OCH$_3$: 3.80 (s), CH$_2$: 3.56 (s), 8-H, 10-H: 3.1 (superimposed), 19-H$_3$, 17-H$_3$: 1.84 (dd), 1.77 (s), 18-H$_3$: 1.15 ppm (d, J=7 cps).

RESULTS

By base-catalyzed transesterification of resiniferatoxin, resiniferonol-9,13,14-orthophenylacetate (**III**, see fig. 1) and the corresponding methyl ester **II** were obtained. **II** exhibited a retention time in glc analysis identical with that of authentic homovanillic acid methyl ester ((4-hydroxy-3-methoxyphenyl) acetic acid methyl ester, **II**). Esterification of **III** with homovanillic acid afforded an ester with spectral data fully identical with resiniferatoxin. The correct structure of resiniferatoxin is therefore **I**, i.e., resiniferonol-9,13,14-orthophenylacetate-20-(4-hydroxy-3-methoxy)phenyl acetate. The structure of resiniferatoxin proposed previously (1) to be the isomeric 20-(5-hydroxy-3-methoxyphenyl)acetate was derived solely from spectral data and was not finally confirmed by partial synthesis (see below). Further proof of structure **I** was achieved by obtaining **IV**, either by treatment of **I** with diazomethane or by esterification of **III** with homoveratric acid (3,4-dimethoxyphenyl)acetic acid. Both reaction products exhibited identical spectral data. The alternative compound **V**, obtained by partial synthesis from **III** and (3,5-dimethoxyphenyl)acetic acid, differed clearly from **IV** in its spectral data. Starting from **III**, further resiniferonol-20-esters were synthesized with phenylacetic, benzoic, hexanoic and acetic acid, respectively, (**VI–IX**, fig. 1). For irritant activities see table 1.

For preparation of the parent alcohol resiniferonol (**XI**), the orthoester **III** had to be cleaved. As demonstrated by Sakata et al. (12) huratoxin, an unsaturated fatty acid 9,13,14-orthoester of 5-hydroxyresiniferonol-6α,7α-oxide, was hydrolyzed by refluxing with aqueous ethanol containing 2 M HCl for 7 hours (30% yield). However, under these drastic conditions the 6,7-oxirane ring in the diterpene parent was also hydrolyzed. Under milder conditions, lower temperature and shorter reaction time, resiniferonol-14-phenylacetate (**X**, see fig. 2) was obtained (35% yield) from **III**. Presence of a 14-ester group is indicated by the paramagnetic shifts of 14-H from 4.23 to 5.56 ppm, 8-H from 3.1 to 3.7 ppm and of the methylene protons of the phenylacetate from 3.19 to 3.83 ppm in the nmr-spectrum of **X**, as compared to the starting material **III**. Furthermore, in the ir-spectrum of **X** the stretching vibration band of an ester carbonyl group at 1730 cm^{-1} is apparent. Mild alkaline conditions of transesterification of **X** afforded the water-soluble parent alcohol resiniferonol (**XI**). The mass spectra of **X** and

I   : R = CO - CH$_2$- ⟨OCH$_3$⟩OH   (resiniferatoxin)

III   : R = H

IV   : R = CO - CH$_2$ - ⟨OCH$_3$⟩OCH$_3$

V   : R = CO - CH$_2$ - ⟨OCH$_3$⟩OCH$_3$

VI   : R = CO - CH$_2$ - ⟨ ⟩

VII : R = CO - ⟨ ⟩

VIII : R = CO - (CH$_2$)$_4$ - CH$_3$
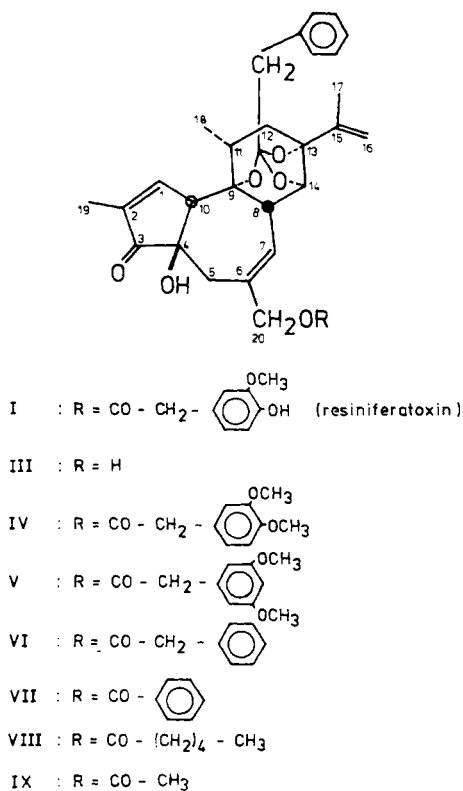
IX   : R = CO - CH$_3$

FIG. 1.   Resiniferatoxin   (I)   and   various
other esters of resiniferonol-9,13,14-
orthophenylacetate (III).

X   : R^1 = CO - CH$_2$- ⟨ ⟩   , R^2 = H

XI   : R^1 = R^2 = H (resiniferonol)

XII : R^1 = R^2 = CO - CH$_3$
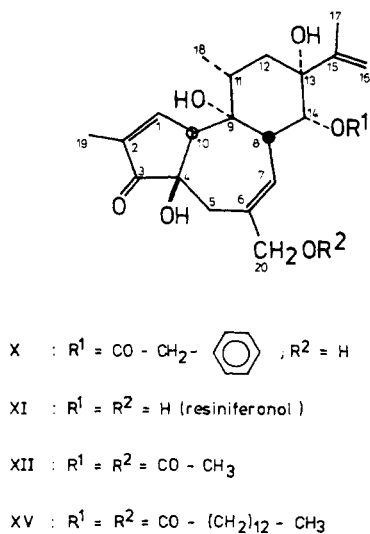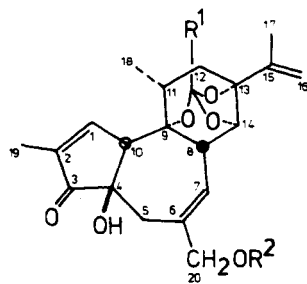
XV : R^1 = R^2 = CO - (CH$_2$)$_{12}$ - CH$_3$

FIG. 2.   Resiniferonol, a resiniferonol-
14-ester and -14,20-diesters.

XIII : R^1 = CH$_3$, R^2 = H

XIV : R^1 = CH$_3$, R^2 = CO - CH$_2$ - (3,5-dimethoxyphenyl)

XVI : R^1 = (CH$_2$)$_{12}$ - CH$_3$; R^2 = CO - (CH$_2$)$_{12}$ - CH$_3$

XVII : R^1 = (CH$_2$)$_{12}$ - CH$_3$; R^2 = H

XVIII R^1 = (CH$_2$)$_{12}$ - CH$_3$; R^2 = CO - CH$_2$ - (3,5-dimethoxyphenyl)

FIG. 3. Resiniferonol-9,13,14-orthoacetate, resiniferonol-9,13,14-orthotetradecanoate and 20-esters thereof.

XI (electron impact) exhibited the fragment ions M$^+$–H$_2$O only and further fragments thereof, whereas, when the field desorption technique was used, the ms of XI exhibited also the parent ion at m/e 364.

When XI was esterified with acetic anhydride and tetradecanoic acid chloride, the corresponding resiniferonol-14,20-diesters XII and XV were obtained. Mild acidic treatments of XII and XV, respectively, with perchloric acid in methanol (or exposure to heat) led to the formation of the orthoesters XIII and XVI (upfield shift of 14–H from appr. 5.55 to 4.28 ppm; upfield shift of the acetyl protons in XIII to 1.67 ppm). Under these conditions the acetyl group at C–20 in compound XII was also cleaved. When the C–20 acid group in XV was removed by subsequent base-catalyzed transesterification resiniferonol-9,13,14-orthotetradecanoate was obtained (XVII, for structures see fig. 3). Re-esterification of these resiniferonol orthoesters with (3,5-dimethoxyphenyl)acetic acid (downfield shifts of protons 20–H$_2$ from appr. 4.05 to 4.55 ppm) afforded the aromatic esters resiniferonol-9,13,14-orthoacetate-20-(3,5-dimethoxyphenyl)acetate (XIV) and resiniferonol-9,13,14-orthotetradecanoate-20-(3,5-dimethoxyphenyl)acetate (XVIII), respectively. Their ID$_{50}$-values are noted in table 1.

DISCUSSION

Influence of the acid moiety in **20**-*position on the biological activity of resiniferonol esters.* Recently, a number of differently substituted aromatic and aryl aliphatic 20-esters of resiniferonol-9,13,14-orthophenylacetate (III) were synthesized and tested for irritant activity (8) on the mouse ear. They exhibited only weak or no irritant activity. Part of this work was based upon the structure for resiniferatoxin proposed previously which had to be revised according to the results described here of the partial synthesis of this highly irritant *Euphorbia* factor.

As already demonstrated (8, 11), cleavage of the 20-ester group in I, yields III with dramatically decreased irritant activity. This is suggestive of a particular role for the specific structure of the 20-ester group in I. For example, it is well known that phenolic compounds related to that structural type of the 20-ester

TABLE 1. Irritant activity of 20-esters of resiniferonol (**XI**), determined by the mouse ear assay, read 5 and 24 hours after application of the compound and expressed by ID_{50}-values (nmole/ear); reference compound TPA (12-O-tetradecanoylphorbol-13-acetate) ID_{50}^5: 0.012 nmoles/ear; ID_{50}^{24}: 0.016 nmoles/ear.

Compound/Factor	ID_{50}^5	ID_{50}^{24}
Resiniferatoxin (**I**)...	1.6×10^{-5}	>20
Resiniferatoxin methylether (**IV**).........................	4.7×10^{-4}	>20
Resiniferonol-9,13,14-orthophenylacetate-20-(3,5-dimethoxyphenyl) acetate **V**...........................	6.2×10^{-4}	>20
Resiniferonol-9,13-14-orthophenylacetate-20-phenylacetate (**VI**)....................................	1.7×10^{-3}	>10
Resiniferonol-9,13,14-orthophenylacetate-20-benzoate (**VII**)..	1.2×10^{-2}	>10
Resiniferonol-9,13,14-orthophenylacetate-20-hexanoate (**VIII**).......................................	8.9×10^{-2}	>10
Resiniferonol-9,13,14-orthophenylacetate-20-acetate (**IX**)...	>10	>10
Resiniferonol (**XI**)....................................	>100	>100
Resiniferonol-9,13,14-orthophenylacetate (**III**)............	1.0	>20
Resiniferonol-9,13,14-orthoacetate (**XIII**).................	>100	12.9
Resiniferonol-9,13,14-orthotetradecanoate (**XVII**).........	>30	0.8
Resiniferonol-9,13,14-orthotetradecanoate-20-tetradecanoate (**XVI**).................................	>60	>60
Resiniferonol-9,13,14-orthoacetate-20-(3,5-dimethoxy-phenyl) acetate (**XIV**)............................	2.6×10^{-2}	> 5
Resiniferonol-9,13,14-orthotetradecanoate-20-(3,5-dimethoxyphenyl) acetate (**XVIII**)..............	> 5	> 5
Resiniferonol-14-phenylacetate (**X**)......................	>100	>100
Resiniferonol-14,20-diacetate (**XII**)......................	>20	>20
Resiniferonol-14,20-ditetradecanoate (**XV**)...............	>60	>60
(4-Hydroxy-3-methoxyphenyl) acetic acid methyl ester (**II**)...	25	>100

group may be skin irritants [for a recent review see (13)]. 3-Alkyl derivatives of catechol are the highly irritant factors from Anacardiaceae (e.g. the well known *Toxicodendron radicans* or poison ivy) (14) and 5-alkyl derivatives of resorcinol with strong skin irritating effects have been isolated from Protaceae, e.g. (15, 16). Also, the characteristic phenolic acid moiety present in **I**, in the form of its methyl ester, exhibits weak irritant activity (see table 1). It may be speculated, therefore, that the catechol part of the 20-ester group in **I** might contribute specifically to its extremely high irritant activity on skin. Indeed, the methylation product **IV** shows only a slight reduction of biological activity (see table 1).

The position of the substituents on the aromatic ring does not seem to influence the irritant activity of the molecule considerably since resiniferonol-9,13,14-orthopenylacetate-20-(3,5-dimethoxyphenyl)acetate (**V**) is as active as the isomeric 3,4-dimethoxyphenyl)acetate **IV**. The important role of the phenolic or phenol ether moieties in the 20-ester group of **I** is also evident when the irritant activities of compounds **I** and **VI** are compared. **VI** with an unsubstituted phenyl acetic acid moiety is about 100 times less irritant than **I**. The 20-benzoate **VII** is even less active. However, both compounds **VI** and **VII** are still more active than the most active phorbol ester, *i.e.*, TPA (see table 1). Aliphatic acyloxy moieties at the 20-position lead to substantial loss of irritant activity. Thus, the 20-hexanoate **VIII** is 5000 times less active than **I**, and the 20-acetate **IX** has practically lost irritant activity.

In summary, it may be concluded that the extreme irritant activities of the natural products resiniferatoxin (**I**) and tinyatoxin (with 20-(4-hydroxyphenyl)-acetoxy group (8) are related to their specific structures as 20-esters with phenolic

phenylacetic acids. Esterification of the phenolic hydroxyl groups in **I** and in tinyatoxin with acetic acid (8) or etherification of the phenolic hydroxyl group in **I** with methanol have no influence on the high irritant activity of these compounds.

Influence of the orthoester group and of its acid moiety on the biological activity of resiniferonol esters. To evaluate the role of the acid moiety esterified with the hydroxyl groups 9α, 13α and 14α, cleavage of the orthoester group in two stages was essential. The product of the first stage, resiniferonol-14-phenylacetate (**X**), as well as that of the second stage, resiniferonol (**XI**), do not exhibit measureable irritant activity. Also, resiniferonol-14-phenylacetate-20-(4-hydroxy-3-methoxy) phenyl acetate exhibits only very weak irritant activity compared to **I** (1). These data clearly indicate that, in addition to specific 20-ester groups, the 9α, 13α, 14α-orthoester group also contributes to irritancy. The specific structure of the orthoester group adds to the potency of the compound as may be seen by comparison of **V** and **XIV**.

12-Deoxyphorbol esters carrying at C–13 a *long chain* aliphatic acyloxy group, e.g., 12-deoxyphorbol-13-tetradecanoate represent highly irritant and cocarcinogenic compounds (17), whereas 12-deoxyphorbol-13-acetate (prostratin) (18) exhibits practically no irritant activity on the mouse ear. Therefore, resiniferonol (**XI**) was esterified with both acetic and tetradecanoic acid to obtain resiniferonol-14,20-diacetate (**XII**) and resiniferonol-14,20-ditetradecanoate (**XV**), respectively. The readily obtained aliphatic orthoesters with free hydroxyl groups at C–20, i.e., resiniferonol-9,13,14-orthoacetate (**XIII**) and resiniferonol-9,13,14-orthotetradecanoate (**XVII**) do not exhibit irritant activity on the mouse ear 5 hours after application. However, both esters show weak but reproducible activity 24 hours after application, **XVII** being about 10 times more active than resiniferonol-9,13,14-orthoacetate (**XIII**). However, **XVII** is still less irritant than TPA by a factor of 50 (see table 1). Esterification of the 20-hydroxyl group of the resiniferonol-orthoesters **XIII** and **XVII** with (3,5-dimethoxyphenyl)acetic acid only leads to to the esters with moderate (**XIV**) or no irritant activity (**XVIII**), as compared to **V**.

Hence it may be concluded that derivatives of resiniferonol with saturated fatty acids in 9α, 13α and 14α-position exhibit only weak or no irritant activity, although these acids are entities important for irritant activity of diterpene esters of the tigliane- and ingenane-type (4, 5).

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