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NEW IRRITANT DITERPENE-ESTERS FROM ROOTS OF STILLINGIA SYLVATICA L. (EUPHORBIACEAE)

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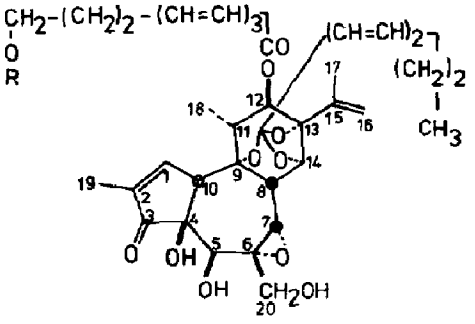
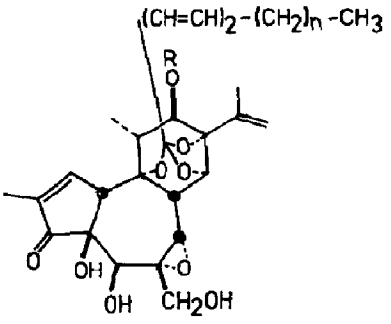
ABSTRACT: From roots of Stillingia sylvatica (Euphorbiaceae) eight more or less irritant Stillingia factors S_1 - S_8 were isolated and identified as diterpene esters of the daphnane and tigliane types carrying saturated, polyunsaturated or hydroxlated fatty acids.

The sap of roots of Stillingia sylvatica L., indigenous to Northern America (Queen's delight) is wellknown to be toxic and irritating, causing swelling and inflammations on skin and mucous membranes[1]. Until several years ago, a vitamin enriched tonic containing an alcoholic extract of Stillingia sylvatica was commercially available in the United States.

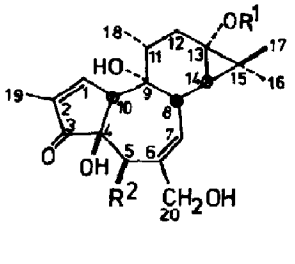
The fractionation procedure for the isolation of irritant compounds from roots was guided by the assay for irritant activity on the mouse ear[2]. Roots were homogenized, extracted with methanol and the methanolic extract partitioned between ethyl acetate and water. The ethyl acetate extract was subjected to countercurrent distributions by two O'Keeffe distributions (z=100 elements, systems: i. carbon tetrachloride/methanol/water=2/1/0.15 and ii. petroleum ether/methanol/water=15/10/0.5) followed by a Craig distribution in the latter system (z=1020 elements, n=3000 transfers). Preparative thin layer chromatography (TLC) and in some cases HPLC of the fractions obtained afforded irritant factors.



1: R=H
2: R=CO-$(CH_2)_{12}$-CH_3
3: R=CO-CH=CH-$(CH_2)_{11}$-CH_3



4: R=H; n=2
5: R=CO-$(CH=CH)_3$-$(CH_2)_3$-$OCOC_{13}H_{27}$; n=4
6: R=CO-$(CH=CH)_3$-$(CH_2)_3$-$OCOC_{14}H_{27}$; n=4
7: R=COC_6H_5; n=4 (gnidilatidin)



8: R^1=$COCH_3$; R^2=H (prostratin)
9: R^1=CO-$(CH_2)_{14}$-CH_3; R^2=OH

Stillingia factor S_1 (1) was isolated from the very hydrophilic fraction obtained after Craig distribution; ms: parent ion m/e 680; uv (methanol): 231 (31500), 305 nm (28500); nmr: see chart 1; for irritancy and yield of all factors see Table 1. The nmr-data indicate presence of a derivative of the diterpene hydrocarbon daphnane[+)] with hydroxyl groups at C-atoms 9, 13 and 14 forming an orthoester with an unsaturated fatty acid. Representative compounds of this class of toxic natural products have been isolated and characterized by X-ray analysis in recent years, e.g. daphnetoxin from Daphne mezereum[6] and huratoxin from Hura crepitans[7]. The structure of the fatty acids in Stillingia factor S_1 was elucidated following transesterification of S_1 with sodium methoxide in methanol affording as an acid moiety ω-hydroxy-2,4,6-decatrienoic acid methyl ester; ms: parent ion m/e 196; uv: $\lambda_{max}(\varepsilon_{max})$: 300 (17000); nmr ($CDCl_3$): 1 olefinic H: 7.25 (dd); 3 olefinic H: 5.7-6.7, OCH_3: 3.76, CH_2OH: 3.68 ppm (t). Upon acetylation of the methyl ester a product was obtained whose nmr-spectrum shows a downfield shift of the triplet to 4.1 ppm and the acetyl signal at 2.05 ppm. - As the diterpene moiety (4) was obtained: ms: parent ion m/e 516; uv: $\lambda_{max}(\varepsilon_{max})$: 231 nm (31300); nmr: 1-H: 7.6 (m); 1 olefinic H: 6.69 (dd); 3 olefinic H: 5.5-6.3; 16-H_2: 5.10 (s, br.); 14-H: 4.76 (d, J=2 Hz); 5-H: 4.26 (s); 20-H_2: 3.86; 12-H: 3.90 (s); 10-H: 3.8-3.9 (superimposed); 8-H: 3.76 (d, J=2 Hz); 7-H: 3.53 (s); 11-H: 2.5 (q); 17-H_3: 1.88 (s); 19-H_3: 1.80 (dd); 18-H_3: 1.16 ppm (d). The nmr- and ms-data indicate that in (1) cleavage of the ester group in position 12 takes place leaving the diterpene moiety (4) with an octadienoic acid in 9,13,14-orthoester position. The nmr-data are in good agreement with those published for a transesterification product. ("tetrol 7", ms: m/e 544)[8] obtained from gnidilatidin (7) carrying a 9,13,14-ortho-2,4-decadienoic acid on the same parent alcohol, i.e. 5β,12β-dihydroxyresiniferonol-6α,7α-oxide (for nomenclature see[9]). The compounds obtained and their data lead unambiguously to structure 1 for Stillingia factor S_1.

Stillingia factors S_2/S_3: ms: parent ions m/e 902 and 890, prominent peak m/e 499, were not separable by TLC or HPLC (reserved phase). The nmr-data are very similar to those of factor S_1 except a downfield shift of the characteristic triplet to 4.1 ppm indicating esterification of the ω-hydroxydecatrienoic acid in 9,13,14-orthoester position.

By treatment with sodium methoxide in methanol as acid moieties a mixture of saturated and unsaturated acid methyl esters were obtained. Separation by prep. TLC affords again ω-hydroxy-2,4,6-decatrienoic acid methyl ester (data above) as well as a mixture (ms: M^+ 254 and 242) of tetradecanoic acid methyl ester (identiefied by GLC) and pentadecenoic acid methyl ester. The latter yields upon catalytic hydrogenation pentadecanoic acid methyl ester (GLC). Uv-absorbtion (maximum at 224 nm) indicate presence of a conjugated double

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[+)] in analogy to the nomenclature for the diterpene hydrocarbons tigliane and ingenane this new hydrocarbon ("13,15-secotigliane") was called daphnane (e.g.[3,4]). Later on it was detected that Chem. Abstr. has given this term to a hypothetical parent compound[5] of certain alkaloids isolated from the plant family Daphniphyllaceae. Since the authors working on the alkaloids do not use the term daphnane we will continue to use it for the hypothetical diterpene hydrocarbon in the semitrivial nomenclature which proved most useful for all practical purposes.

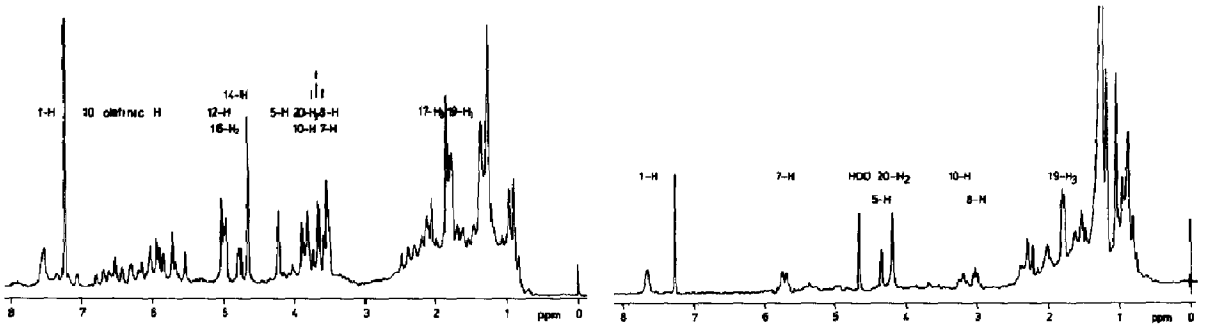

Chart 1: 90 MHz nmr-spectra of Stillingia factors S_1 and S_8, both in $CDCl_3$ + D_2O with TMS (δ=0.00 ppm) as internal standard.

bond in the unsaturated C-15 acid. The data of the two diterpene moieties isolated after transesterification are fully identical with those obtained for Stillingia factor S_1 and compound (4), respectively. Hence, factors S_2/S_3 represent esters of Stillingia factor S_1 with tetradecanoic and 2-pentadecenoic acid being esterified with the primary hydroxy-group of the ω-hydroxy-decatrienoic acid (structures 2 and 3).

Stillingia factors S_4/S_5 (5,6; not separable by TLC or HPLC): ms: parent ions m/e 930 and 918, prominent fragment peak m/e 527; exhibit identical nmr-spectrum as factors S_2/S_3. Comparison of the two prominent fragment ions in the ms-spectra 2/3 and 5/6 (i.e. m/e 499 and 527) suggests presence of C_2H_4-homologous acids. The fragmentation indicates cleavage of the molecular ion at position 12 to yield the corresponding acids and the intensive fragment ions m/e 499 and 527. Thus it may be concluded that the C_2H_4-homology refers to the acid moiety in the orthoester position, i.e. in Stillingia factors S_4/S_5 OH-9, -13 and -14 carry decadienoic acid. This was proved by transesterification of S_4/S_5 leading to a diterpene moiety with a parent ion 28 mass units higher than that of compound 4 but otherwise identical spectral data. Hence, factors S_4/S_5 represent C_2H_4-homologues of factors S_2/S_3 with respect to the unsaturated 9,13,14-orthoester group (structures 5 and 6).

Stillingia factors S_6 and S_7. S_6 proved to be identical with gnidilatidin (7) as isolated by Kupchan and coworkers[8] from Gnidia latifolia (plant family: Thymelaeaceae). S_7 proved to be identical with prostratin (8) isolated previously from Pimelea prostrata (family: Thymelaeaceae).

Stillingia factor S_8 (9): ms: parent ion m/e 602, nmr: see chart 1, exhibit similar nmr-data as esters of 12-deoxyphorbol such as prostratin (8). Comparison of the nmr-data of prostratin and Stillingia factor S_8 exhibit an upfield shift of the signal for 20-H_2 (0.2 ppm) and a new signal at 4.3 ppm being associated in the daphnane derivatives with 5α-H. Thus it may be concluded that Stillingia factor S_8 represents a 12-deoxy-5β-hydroxy-phorbol-13-hexadecanoate (9). The homologous ester, -13-tetradecanoate was recently isolated from Baliosperum montanum (Euphorbiaceae)[11].

Table 1: Yields and Irritancy of factors isolated from Stillingia sylvatica

Factor	Structure	Yield (%)[a]	Molecular Ion	ID_{50}[b] (NMOLES/EAR)
S_1	1	0.014	680	0.16
S_2/S_3	2/3	0.012	890/902	0.08
S_4/S_5	5/6	0.005	918/930	0.12
S_6	7	0.009	648	0.11
S_7	8	0.003	372(M^+-H_2O)	>100
S_8	9	0.0009	602	0.06

a) by weight of methanol extract

b) ID_{50}= irritant dose 50 determined on the mouse ear[2], positive control: 12-O-tetradecanoylphorbol-13-acetate (TPA), ID_{50}: 0.016 nmoles/ear.

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