Tetrahedron Letters No. 19, pp 1587 - 1590, 1975. Pergamon Press. Printed in Great Britain.

ON THE IRRITANT AND COCARCINOGENIC PRINCIPLES OF HIPPOMANE MANCINELLA.

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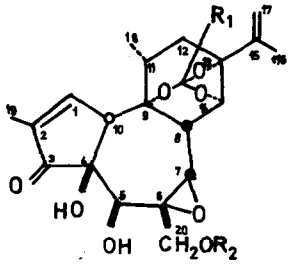
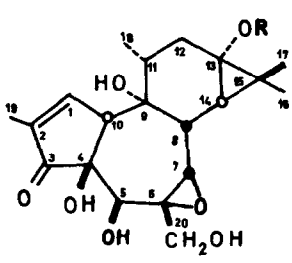
(Received in UK 13 March 1975; accepted for publication 27 March 1975)

The manchineel tree (Hippomane mancinella L., Euphorbiaceae) native to central America and the West Indies, ranks among the most famous poisonous plants in tropical America. The fruits, resembling small apples, and the milky sap in the bark, leaves and twigs are highly toxic to livestock and man. Several attempts have been made to investigate the toxic principles (1,2) but pure active compounds have not been isolated so far (3).

The acetone extract of a methanolic preparation of fresh latex from Hippomane mancinella was subjected to an O'Keeffe distribution to yield an irritant and cocarcinogenic hydrophilic fraction and a non irritant hydrophobic fraction (for assays used see 4). By prep. TLC from the hydrophilic fraction a highly irritant mixture M_x of Hippomane factors (M_1,M_2) and a further Hippomane factor M_3 were isolated; from the hydrophobic fraction a non irritant mixture M_x' was obtained. M_x, M_3 and M_x' are uniform by thin layer chromatography (stain (4): vanillin/sulfuric acid, 110°C).



M_1, M_2; M_x'



M_3

M_1, R_1: $(CH{=}CH)_2$-$(CH_2)_8$-CH_3; R_2: H

M_2, R_1: $(CH{=}CH)_3$-$(CH_2)_8$-CH_3; R_2: H

M_x', R_1: orthoester groups as in M_x,
R_2: CO-$(CH_2)_n$-CH_3, n= 14,16,18,
20,22,24, respectively

M_3, R: CO-$(CH{=}CH)_3$-$(CH_2)_8$-CH_3

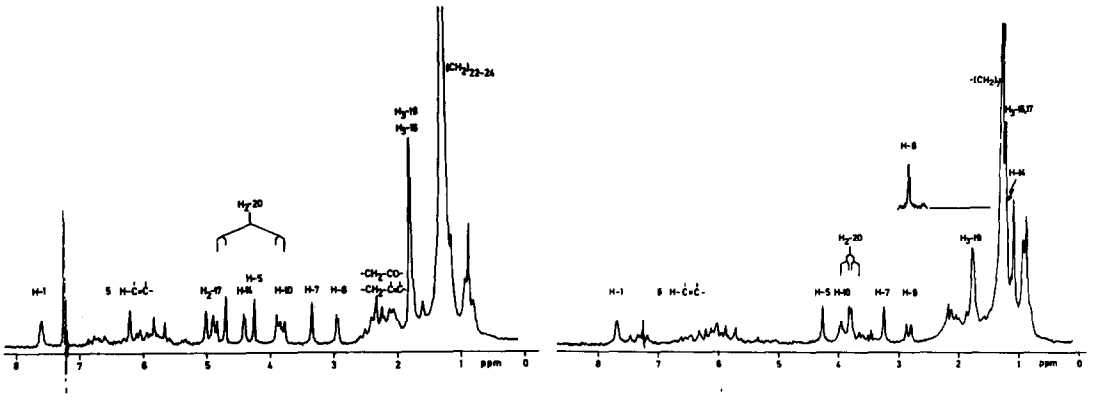
M_x represents a mixture of two esters, M_1 and M_2 which were not separable by chromatographic means; ms: mw 610/584, fragment ions: 360, 342, 317, 233, 207; ir (CH_2Cl_2): 1690 cm^{-1} (CO); uv (CH_3OH): λ_{max}: 194, 240 (sh), 259, 268.5,

+) Dedicated to Prof.Dr.Eugen Müller, Tübingen, on occasion of his 70th birthday.

278.5 nm (ε_{max}: 14500, 20000, 30000, 39000, 31500, average mw: 597); nmr (δ, $CDCl_3$): 5 olefinic protons: 5.5-6.9 ppm, all other signals are identical with those described by Sakata et al. (5) for Huratoxin (from Hura crepitans, Euphorbiaceae). Therefore, M_1 is identical with Huratoxin, the tetradeca-2,4-dienoic acid othoester of a tricyclic diterpene parent alcohol. The new Hippomane factor M_2 is the homologous hexadeca-2,4,6-trienoic acid orthoester of the same parent alcohol (at variance with Huratoxin: uv data, fragment ions m/e: 207 and 233, nmr: average of 5 olefinic protons).

The non irritant M_X' represents a mixture of 20-esters of M_X, since by acid catalyzed transesterification (0.3% $HClO_4$ in CH_3OH) an irritant mixture is obtained with the same spectroscopic properties as M_X. Spectral data of M_X': ms: parent ions m/e: 988/962, 960/934, 932/906, 904/878, 876/850, 848/822; ir (CH_2Cl_2): 1732, 1690 cm^{-1} (CO); uv (n-hexane): data correspond to those of M_X; nmr (chart 1a), differences to the spectrum of M_X: the signal of H_2-20 is shifted downfield, appearing in M_X as s at 3.83 ppm, in M_X' as AB-system at 4.3 ± 0.46 (J_{AB}: 12 hz). Furthermore, in M_X the signal of the aliphatic CH_2-groups at 1.27 ppm corresponds to approximately 7 CH_2-groups, whereas in M_X' this signal at 1.3 ppm corresponds to 22-24 CH_2-groups. Only two signals may be assigned to hydroxyl protons exchangeable with D_2O, all other signals in the nmr spectrum of M_X' correspond to those of M_X. Transesterification of M_X' with 10^{-2} M $NaOCH_3/CH_3OH$ yields a mixture of methyl esters which was resolved by GC, the individual compounds identified and determined quantitatively: methyl hexadecanoate (8.7%), octadecanoate (12.4%), eicosanoate (9.8%), docosanoate (8.0%), tetracosanoate (29.9%) and hexacosanoate (31.2%). Hence M_X' comprises a mixture of 20-esters of M_1 + M_2 with long chain fatty acid residues representing the typical structures (4) of cryptic irritants and cocarcinogens.

The spectral data of the new Hippomane factor M_3 show similarities with those of M_1 but the presence of another new diterpene parent is obvious: ms: mw 612, base peak: m/e= 233; ir (CH_2Cl_2): 3520, 3380 (OH), 1685 (CO), 1610 cm^{-1} (C=C); uv (CH_3OH): λ_{max}: 194, 253, 306.5 nm (ε_{max}: 12000, 12000, 27000); nmr (δ, $CDCl_3$, see also chart 1b): H-1: 7.68 (s, broad); 6 olefinic protons: 5.5-7.5; H-5: 4.26 (s); H-10: 3.95 (s, broad); H_2-20: 3.805 ± 0.047, J_{AB}: 12 hz; H-7: 3.25



a b

chart 1: 90 MHz spectra of a) mixture M_x; b) Mancinellin (factor M_3), both in $CDCl_3$ + D_2O with TMS (δ = 0.00 ppm) as internal standard.

(s); H-8: 2.83 (d, 7 hz); H_3-19: 1.78 (s, broad); $-(CH_2)_7-$: 1.28 (s); H_3-16,17: 1.21, 1.09 (s); H_3-18: 0.92 (d, 5 hz); 4 OH (exchangeable with D_2O): 5.3, 4.14, 3.82 and 3.48 ppm. As compared to the spectrum of M_x the signals for H-14 at 4.42 ppm, H_3-16 at 1.8 ppm and H_2-17 at 5.02 and 4.89 ppm are missing, whereas the signals for 2 geminal methyl groups are present as in the spectrum of phorbol (4). Decoupling experiments demonstrate that H-8 couples with a cyclopropane-proton (H-14) at 1.21 ppm (chart 1b). With the exception of the signals of 4 OH-groups, all other signals correspond to those in the spectrum of M_x. Therefore, the nmr-spectrum indicates, at variance with Hippomane factors M_1 and M_2, the presence of a tetracyclic parent alcohol with a cyclopropane ring and a tertiary hydroxyl group esterified with hexadeca-2,4,6-trienoic acid (m/e= 233, 6 olefinic protons, $-(CH_2)_7-$). Positioning of the ester residue at OH-13 is demonstrated by acetylation of M_3 with Ac_2O/Py yielding a 5,20-diacetate (nmr, ms). α-configuration of the epoxide ring is proposed because in related phorbol-6α,7α-oxides the dihedral angle between H-7 and H-8 is 90° (6). The configuration at C-5 is assumed to be identical with that in Huratoxin. The stereochemistry of all other groups may be derived from that in phorbol. Thus, M_3 represents a 13-hexadeca-2,4,6-trienoate of 12-deoxy-5β-hydroxy-phorbol-6α,7α-oxide, for which the name "Mancinellin" is suggested.

On the mouse ear M_x shows an irritant dose 50 (ID_{50}) of 0.02 µg/ear, whereas factor M_3 is about 10 fold less active with an ID_{50} of 0.15-0.20 µg/ear.

From the biogenetic point of view it is interesting to see that the latex of Hippomane mancinella contains both, diterpene esters of the tetracyclic tigliane type (in M_3) and of the tricyclic daphnane type (in M_x). Further, it is remarkable that, contrary to the diterpenes of the macrocyclic duvane and the tricyclic rhamnifolane type with their isopropenyl group at C-14 (7), daphnane and its derivatives carry the isopropenyl group at C-13. Therefore the latter cannot be visualized simply as product of an incomplete condensation of geranyl-geranyl pyrophosphate according to the isoprene rule as in case of rhamnifolane and duvane (7). Rather, the biogenetic precursors of daphnane might be tigliane type diterpenes: for example, by hydroxylation of Mancinellin (M_3) at C-14, oxidative ring opening (for chemical analogy see formation of bisdehydrophorbol-12,20-dieacetate from phorbol-12,20-diacetate, loc.cit. 4) and reduction of the 13,14-ketolester group to a 13α,14α-glycolester group with a 13β-isopropenyl group may be generated. In diterpenes of this type the hydroxyl groups in 9α,13α- and 14α-position may be spatially related (see also 5) in such a manner as to allow easy formation of an orthoester group if one of them is esterified. Recently, this was demonstrated by the facile conversion of proresiniferatoxin to resiniferatoxin (8).

ACKNOWLEDGEMENTS

We wish to thank Miss B.Pieruschka, Miss I.Jung and Mr. J.Fischer for skilled technical assistance. The cooperation in collecting the latex by Mr. I.G. Koblick, Mr.M.E.Stephey, Dr.A.E.Damman and Dr.E.L.Towle from Virgin Islands, Ecological Research Station, Carribean Research Institute, St.John, U.S.Virgin Islands is gratefully acknowledged.

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