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ISOLATION AND STRUCTURE OF INSECTICIDAL COMPONENTS FROM MAMMEA AMERICANA L.

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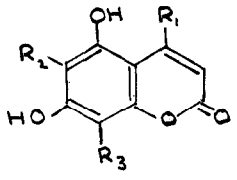
The light petroleum extract of the seeds of Mammea americana L. is insecticidally active[1]. In recent work we have isolated four 4-alkyl coumarins (I-IV)[2] and five 4-phenyl coumarins (V-IX)[3]. Mammein of the literature[4] (I containing III as impurity)[2] has been claimed to be the insecticidal component of the seeds[4] but no test results have been given to support this. Screening of the crystalline compounds (I-IX) against mustard beetles has, in fact, shown that none of these have activity approaching that of the mother liquors from their separation.

Search for the active components has continued using column and preparative layer chromatography, and has resulted in isolation of a colourless crystalline substance, m.p. 50-53°, more toxic to mustard beetles than the most active separation residues from which it was isolated.‡ By combination of spectroscopic and chemical information this



(I)(B/BA)[a]	R_1 =	n-Pr	; R_2 = $Me_2C{:}CHCH_2$.	; R_3 = Me_2CHCH_2CO.
(II)(B/BB)	R_1 =	n-Pr	; R_2 = $Me_2C{:}CHCH_2$.	; R_3 = EtMeCHCO.
(III)(B/BC)[b]	R_1 =	n-Pr	; R_2 = $Me_2C{:}CHCH_2$.	; R_3 = PrCO.
(IV)(C/BB)	R_1 =	n-Am	; R_2 = $Me_2C{:}CHCH_2$.	; R_3 = EtMeCHCO.
(V)(A/AA)[c]	R_1 =	Ph	; R_2 = $Me_2CH.CH_2CO$.	; R_3 = $Me_2C{:}CHCH_2$.
(VI)(A/BA)	R_1 =	Ph	; R_2 = $Me_2C{:}CHCH_2$.	; R_3 = Me_2CHCH_2CO.
(VII)(A/AB)	R_1 =	Ph	; R_2 = EtMeCHCO.	; R_3 = $Me_2C{:}CHCH_2$.
(VIII)(A/BB)	R_1 =	Ph	; R_2 = $Me_2C{:}CHCH_2$.	; R_3 = EtMeCH.CO.

[a] major constituents of mammein,[1,4] [b] minor constituent of mammein,[1,4]

[c] mammeisin.[6]

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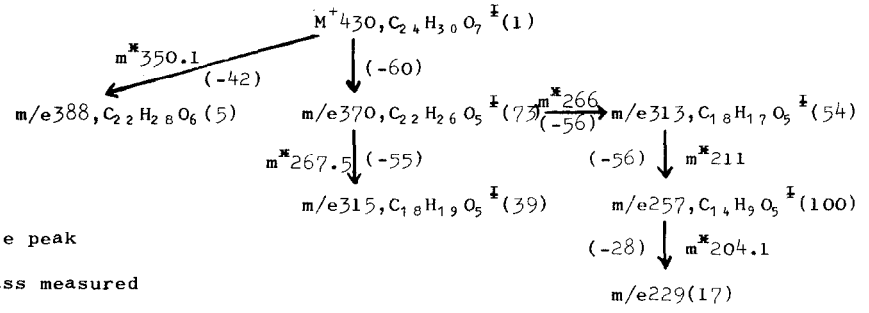
‡ A similar insecticidal product was isolated from M. Africana seeds

substance has been shown to be a mixture of (Xa) and (Xb), though despite intensive efforts it has not proved possible to separate the two closely related isomers(1:2).

The substance (X) was optically active [R.D. (c., 0.04%, ethanol 25°) $[\Phi]_{340}$ -1180° $[\Phi]_{300}$ +753°, $[\Phi]_{240}$ -1075°] and both accurate mass-measurement and combustion gave $C_{24}H_{30}O_7$ as the molecular formulae for the two components. It had λ_{max}(0.01N-HCl)222.2 (log ε 4.50), 293.7(4.39), 320(4.19)nm; λ_{max}(0.01N-KOH) 225(4.25), 257.8(4.06), 332.4 (4.57)nm., indicating a 4-alkyl-5,7-dihydroxycoumarin with an 8-acyl substituent.[2]

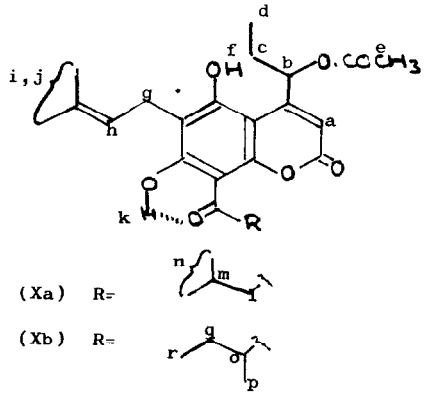
Major mass-spectral fragmentations are summarised below:



m* = metastable peak

‡ indicates mass measured

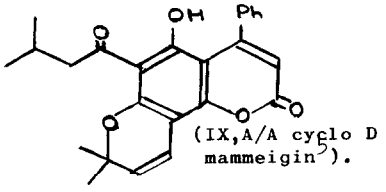
Loss of ketene (M-42) and acetic acid (M-60) from the molecular ion suggest the presence of an acetate group, in agreement with a maximum at 1745 cm^{-1} in the infrared (mull). Fragmentation of the ion m/e 370 by loss of a butenyl radical to give m/e 315, and by loss of a butyl radical to give m/e 313 followed by loss of butene to give m/e 257, are characteristic of compounds possessing 3-methylbut-2-enyl, and 2-methylbutyryl or 3-methylbutyryl substituents.[2,3]



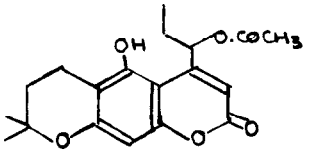
a,	τ 3.72(1)s.		(Xa)
b,	3.50(1)dd. J3Hz,8Hz		
c,	8.2-8.6(2)m.	k,	τ -4.64s.
d,	9.00(3)t. J7Hz	l,	6.85d. J7Hz
e,	7.81(3)s.	m,	7.80m.
f,	2.73(1)s.	n,	8.98d. J7Hz
g,	6.53(2)d. J7Hz		(Xb)
h,	4.80(1)m.		
i)	8.14(3)s.	k,	τ -4.59s.
j)	8.18(3)s.	o,	6.15m.
		p,	8.76d. J7Hz
		q,	8.2-8.6m.
		r,	9.02t. J7Hz

Chemical confirmation of the presence of 2-methyl- and 3-methylbutyryl side chains in (X) was provided by oxidative stripping of 2-methyl- and 3-methylbutyric acids using hydrogen peroxide, (reliability checked with coumarins of established structure), and

the acids were separated and identified by gas-liquid chromatography as the isopropyl esters[7]. The insecticidal substance (X) formed, on methylation, the expected mixture of dimethyl ethers (n.m.r.), m.p. 88-90°, and when boiled with formic acid was deacylated and cyclised to the chromone (XI) m.p. 235-238°. The structure of the latter is based on mass-spectral fragmentation, spectrophotometric Gibbs test, and n.m.r. data.



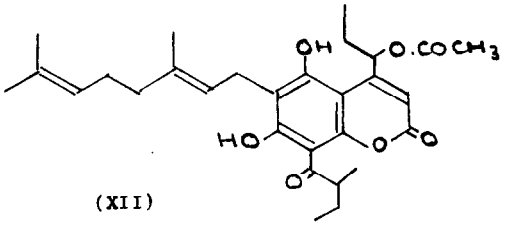
(IX,A/A cyclo D mammeigin[5]).



(XI)

N.m.r. examination of the insecticide mixture (Xa and Xb) is in complete agreement with the information given above and confirms the presence of two types of acyl substituent. Assignments are presented above and proton relationships have been confirmed by double resonance methods.

In further examination of the insecticidal effectiveness of (X) it has been found that the group of compounds (I)-(X) are all uncouplers of oxidative phosphorylation at below 0.5 µg/ml. Substance (X) uncouples at 0.05 µg/ml. and shows substantially greater toxity to houseflies (topical and injection) than compounds (I)-(IX). Recently there appeared a report[8] of the isolation of an antibacterial, acetylated coumarin of related structure, surangin B (XII), from Mammea longifolia roots. In the light of the above results, insecticidal effectiveness might be predicted for the structure. Surangin B was found to uncouple oxidative phosphorylation, to be toxic to mosquito larvae, and to be more active than (X) to houseflies by both methods of test.



(XII)

It is known that coumarin types /- e.g. (V)7[6] are distributed in the pulp and peelings, as well as in the seed, of M. americana. Although much valued as an edible fruit, the flesh has long been suspected of being toxic[1] and connexions between this

and the uncoupling activity of the contained coumarins deserve investigation.

Dr. M. Elliott and and Mr. P. Needham (Rothamstead Experimental Station) provided the testing against mustard beetles. Dr. C.B.C. Boyce (Shell Research Ltd., Woodstock Agricultural Research Centre) suggested to us that these compounds might possess uncoupling activity and the determinations of this, and the other insecticidal data, were made by his colleagues, Dr. J.F. Donnellan and Mrs. N. McFarlane respectively. Dr. A. McCormick carried out a preliminary mass-spectral examination and Dr. B.S. Joshi kindly provided us with a comparison specimen of surangin B. We appreciate the efforts of A.G. Kenyon (Tropical Products Institute) and M.H. Gaskins (U.S.D.A. Agricultural Research Service, Mayaguez, Puerto Rico) in keeping us supplied with mammea kernels.

REFERENCES

1. M.P. Morris and C. Pagán, J. Amer. Chem. Soc., 1953, 1489. H.K. Plank, J. Econ. Entomology, 1944, 37, 737; Trop. Agric., 1950, 27, 38.

2. L. Crombie, D.E. Games and A. McCormick, J. Chem. Soc., (C), 1967, 2545; Tetrahedron Letters, 1966, 151.

3. L. Crombie, D.E. Games, and A. McCormick, J. Chem. Soc. (C), 1967, 2553; Tetrahedron Letters, 1966, 145.

4. C. Djerassi, E.J. Eisenbraun, R.A. Finnegan and B. Gilbert, J. Org. Chem., 1960, 25, 2164. C. Djerassi, E.J. Eisenbraun, B. Gilbert, A.J. Lemin, S.P. Marfey, and M.P. Morris, J. Amer. Chem. Soc., 1958, 80, 3686. C. Djerassi, E.J. Eisenbraun R.A. Finnegan and B. Gilbert, Tetrahedron Letters, 1959, (1), 10.

5. R.A. Finnegan and W.H. Mueller, J. Org. Chem., 1965, 30, 2342; Chem. and Ind., 1964, 1065.

6. R.A. Finnegan and C. Djerassi, Tetrahedron Letters, 1959, (13), 11; R.A. Finnegan, M.P. Morris and C. Djerassi, J. Org. Chem., 1961, 26, 1180.

7. G.A. Howard and A.R. Tatchell, J. Inst. Brewing, 1956, 62, 20; F.L. Rigby, E. Sihto and A. Bars, ibid., 1960, 66, 242.

8. B.S. Joshi, Y.N. Kamat, T.R. Govindachari and A.K. Ganguly, Tetrahedron, 1969, 25, 1453.