Extractives of *Mammea americana* L. Part V.[1] The Insecticidal Compounds

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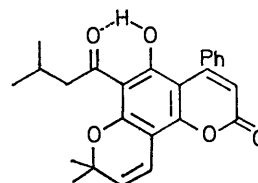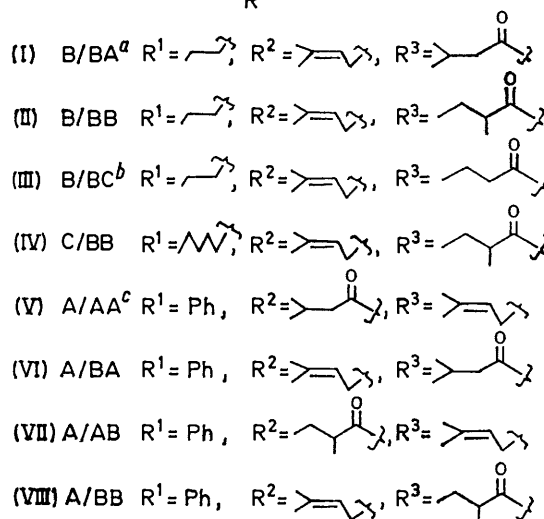
None of the crystalline coumarins reported earlier have insecticidal activity able to account for that of the crude light petroleum extract of *Mammea americana* L. A crystalline mixture, shown to be of the two 4-alkyl-5,7-di-hydroxycoumarins (Xa and b), differing only in the possession of an 8-(3-methylbutyryl) or an 8-(2-methylbutyryl) substituent, has now been isolated. It is more active than the most effective noncrystalline fractions from which it came. There is also evidence in some samples of an 8-(butyryl) member (Xc). The distinguishing feature of the insecticides, relative to the already known extractives (*cf.* Parts I—IV), is the presence of a 4-(1-acetoxypropyl) side-chain. *M. africana* contains a similar insecticidal mixture.

Spectral and chemical findings concerning compounds (Xa—c) are discussed. The insecticidal compounds are uncouplers of oxidative phosphorylation, but this property appears widespread among other coumarins of the plant. From the structural conclusions drawn it appeared that surangin B from *M. longifolia* should be an insecticidal compound; this has been confirmed.

VARIOUS parts of the evergreen tree *Mammea americana* L. (Guttiferae), which is indigenous to the West Indies and tropical America, are insecticidal.[2] Hydrocarbon extracts of the large (*ca.* 40 g) egg-shaped seeds appear most effective, and we have investigated the insecticidal light petroleum extract in our work. Four 4-alkyl-coumarins [(I)—(IV)] and five 4-phenylcoumarins [(V)—(IX)] were isolated (Parts I [3] and II [4]) as crystalline compounds but, relative to the crude extract, none of these showed appreciable toxicity to mustard beetles in topical application tests. Mammein of the literature [5] [(I) containing (II) and (III) as impurity] [3] has been claimed [5] to be the insecticidal component of the seeds but there is no report of biological testing in support. During the work described in Parts III [6] and IV [1] the activities of the crystalline isolates, including mammein, were tested as above; always the activity was negligible relative to the later non-crystalline chromatographic fractions of the extract.

Intensive column and preparative-layer chromatography of the light petroleum extract of *M. americana*, stripped of as much as possible of the crystalline components hitherto identified, was therefore commenced. This eventually resulted in the isolation of a colourless crystalline material, m.p. 50—53°, which was more insecticidally active than the highly active non-crystalline concentrates from which it could be isolated. By the spectral techniques used earlier it was shown to be

a mixture of compound (Xa) and the close relative (Xb): [7] there is also mass spectral evidence of a little (Xc) in some samples.

(I) B/BA[a] R¹ = ⌐⌐; R² = ⌐⌐; R³ = ⌐⌐

(II) B/BB R¹ = ⌐⌐; R² = ⌐⌐; R³ = ⌐⌐

(III) B/BC[b] R¹ = ⌐⌐; R² = ⌐⌐; R³ = ⌐⌐

(IV) C/BB R¹ = ⌐⌐; R² = ⌐⌐; R³ = ⌐⌐

(V) A/AA[c] R¹ = Ph, R² = ⌐⌐; R³ = ⌐⌐

(VI) A/BA R¹ = Ph, R² = ⌐⌐; R³ = ⌐⌐

(VII) A/AB R¹ = Ph, R² = ⌐⌐; R³ = ⌐⌐

(VIII) A/BB R¹ = Ph, R² = ⌐⌐; R³ = ⌐⌐

(IX)[d]

[a] Major constituent of mammein (refs. 3 and 5). [b] Minor constituent of mammein (refs. 3 and 5). [c] Mammeisin (R. A. Finnegan and C. Djerassi, *Tetrahedron Letters*, 1959, 11; R. A. Finnegan, M. P. Morris, and C. Djerassi, *J. Org. Chem.*, 1961, 26, 1180). [d] Mammeigin (R. A. Finnegan and W. H. Mueller, *Chem. and Ind.*, 1964, 1065; *J. Org. Chem.*, 1965, 30, 2342).

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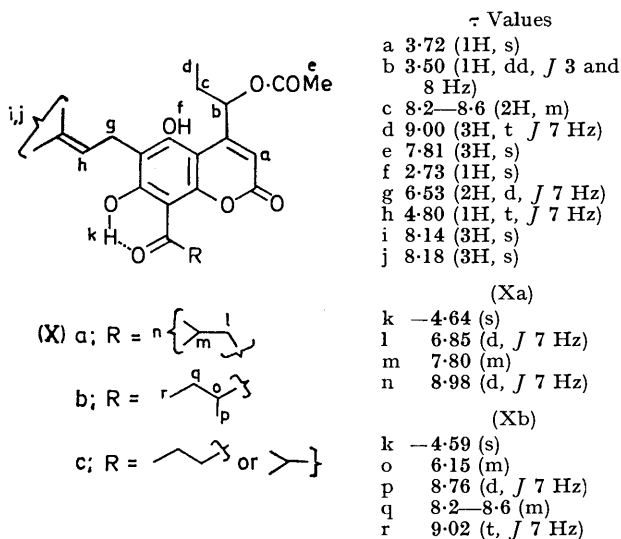[1] Part IV, L. Crombie, D. E. Games, N. J. Haskins, and G. F. Reed, preceding paper.

[2] H. K. Plank, *J. Econ. Entomol.*, 1944, 37, 737; *Trop. Agr.* (*Trinidad*), 1950, 27, 38; *Federal Expt. Puerto Rico, Mayaquez, Bull.*, 1950, 49, 1; M. A. Jones and H. K. Plank, *J. Amer. Chem. Soc.*, 1945, 67, 2266; M. P. Morris and C. Pagan, *ibid.*, 1953, 75, 1489.

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

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

[5] 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, 10.

[6] L. Crombie, D. E. Games, N. J. Haskins, and G. F. Reed, *J.C.S. Perkin I*, 1972, 2241.

[7] Preliminary communication, L. Crombie, D. E. Games, N. J. Haskins, and G. F. Reed, *Tetrahedron Letters*, 1970, 251.

The substance (X) was optically active $\{[\alpha]_D^{24} -28\cdot5°$ (c $0\cdot85$ in ethanol)$\}$ and accurate mass-measurements and combustion analysis led to the molecular formula $C_{24}H_{30}O_7$. The u.v. spectrum $[\lambda_{max.}$ ($0\cdot01\text{N-HCl}$) $222\cdot2$ (log ε $4\cdot50$), $293\cdot7(4\cdot39)$, and 320 nm ($4\cdot19$), $\lambda_{max.}$ ($0\cdot01\text{N-KOH}$) $225(4\cdot25)$, $257\cdot8(4\cdot06)$, and $332\cdot4$ nm ($4\cdot57$)] indicated a 4-alkyl-5,7-dihydroxycoumarin with an 8-acyl substituent.[3] Except for the absorption at 1745 cm^{-1} (assigned to the acetate; see later), the i.r. spectrum $[\nu_{max.}$ (mull) 3380, 1745, 1725, 1625, and 1600 cm^{-1}] was also consistent with a coumarin of the latter type.

τ Values
a $3\cdot72$ (1H, s)
b $3\cdot50$ (1H, dd, J 3 and 8 Hz)
c $8\cdot2$—$8\cdot6$ (2H, m)
d $9\cdot00$ (3H, t J 7 Hz)
e $7\cdot81$ (3H, s)
f $2\cdot73$ (1H, s)
g $6\cdot53$ (2H, d, J 7 Hz)
h $4\cdot80$ (1H, t, J 7 Hz)
i $8\cdot14$ (3H, s)
j $8\cdot18$ (3H, s)

(Xa)
k $-4\cdot64$ (s)
l $6\cdot85$ (d, J 7 Hz)
m $7\cdot80$ (m)
n $8\cdot98$ (d, J 7 Hz)

(Xb)
k $-4\cdot59$ (s)
o $6\cdot15$ (m)
p $8\cdot76$ (d, J 7 Hz)
q $8\cdot2$—$8\cdot6$ (m)
r $9\cdot02$ (t, J 7 Hz)

N.m.r. data for natural mixture.

The major mass-spectral fragmentations of substance (X) (Scheme) provide considerable structural information. Loss of keten ($M - 42$) and acetic acid ($M - 60$) from the molecular ion, and the low abundance of the molecular ion, confirm the presence of an acetate residue. Fragmentation of the ion at 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 characteristics of 5,7-dihydroxy-coumarins possessing 3-methylbut-2-enyl (ozonisation gave acetone) and 2-methylbutyryl or 3-methylbutyryl substituents.[3,4]

Chemical confirmation of the presence of 2-methyl- and 3-methyl-butyryl side chains in (X) was provided by oxidative stripping of 2-methyl- and 3-methyl-butyric acid with hydrogen peroxide. The reliability of the method was checked by use of coumarins of established structure, and the acids were separated and identified as isopropyl esters by g.l.c.[8] Examination of the chromatographs for some specimens of the insecticidal substance (X) showed the presence of small quantities of butyric acid, indicating the presence of a butyryl side-chain. Confirmation of the presence of such a component was provided by mass-spectral

examination of these specimens. A typical case showed, besides the ions normally present, an ion (M^+) at m/e 416 ($C_{23}H_{28}O_7$: measured as $416\cdot1835 \pm 20$; requires $416\cdot1835$) with an abundance of 5% of that of the molecular ion derived from the two isomeric components (m/e 430). The ions at m/e 356 and 341 also showed increased abundance relative to other ions in the spectrum.

The n.m.r. spectrum (CCl_4) of the insecticide mixture confirmed the presence of two types of acyl substituent and resulted in the assignments shown (Xa and b). Proton relationships were confirmed by double resonance. For the 3-methylbut-2-enyl substituent, irradiation of the triplet at τ $4\cdot80$ caused collapse of the doublet at τ $6\cdot53$ to a singlet. Irradiation of the multiplet at τ $6\cdot15$ caused the doublet at τ $8\cdot76$ to collapse and irradiation at τ $8\cdot4$ caused partial collapse of the multiplet at $6\cdot15$ and the triplet at $9\cdot02$ took on singlet form. Irradiation was continued at τ $7\cdot8$ and brought about reduction of the doublet at $6\cdot85$ to a singlet and a similar change in the doublet at $8\cdot98$. This confirms the assignments made for the 2-methylbutyryl and 3-methylbutyryl components. In an investigation of the 4-substituent of the coumarin, irradiation at τ $8\cdot4$ caused partial collapse of the double doublet at $3\cdot50$ and collapse of the triplet at $9\cdot00$ to a singlet.

On methylation the insecticidal substance (X) gave the expected mixture of dimethyl ethers, m.p. 88—90°, and with pyridine–acetic anhydride a low-melting mixture of diacetates. Treatment of (X) with either 98% formic acid or trifluoroacetic acid caused deacylation and cyclisation to the chroman (XI), m.p. 247—249°; this yielded a diacetate. Its identification is based on n.m.r. data as shown, u.v. spectral similarities to other linear chromans (Part IV [1]), and a positive Gibbs reaction (spectrophotometric), together with mass-spectral fragmentation. Loss of acetic acid ($M - 60$) confirmed retention of the acetate, and fragmentation of the ion at m/e 286 by loss of a methyl radical to give m/e 271, or by loss of a butenyl radical to give m/e 231, is characteristic of 2,2-dimethylchromans.[9] In contrast, treatment of (X) with 70% aqueous sulphuric acid at room temperature caused deacylation with isomerisation of (XI) to (XIIa); this formed a diacetate (XIIb). The chroman (XIIa) gave a negative Gibbs test, and n.m.r., i.r., and mass spectral data are in agreement with the structure. U.v. data are consistent with those of other angular chromans (Part III [6]).
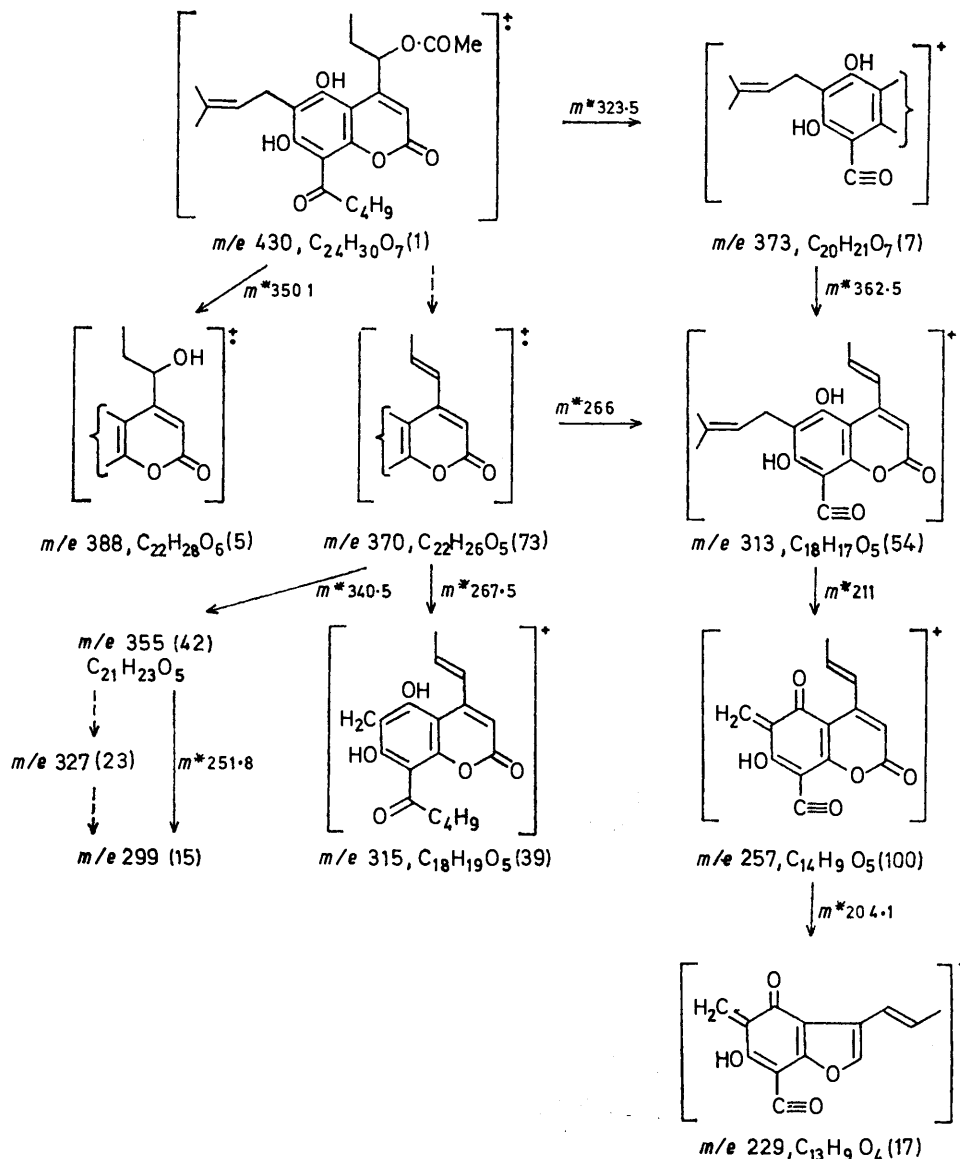
The 8-acylated insecticide (X) was isomerised[5] and deacetylated when treated with methanolic 5% potassium hydroxide, giving the 6-acylcoumarins (XIIIa and b). Spectral data support the structural assignment (see Experimental section). The mixture of side-chain isomers (XIIIa and b) was not C-deacylated when treated with 95% formic acid, but cyclisation and formylation ensued giving crystalline (XIVa and b)

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

[9] B. Willhalm, A. F. Thomas, and F. Gautschi, Tetrahedron, 1964, **20**, 1185; J. R. Trudell, S. D. Sample Woodgate, and C. Djerassi, Org. Mass Spectrometry, 1970, **3**, 753.

(*cf.* Part III [6]). When treated with *m*-chloroperbenzoic acid, (X) gave the mixture of α-(hydroxyisopropyl)-dihydrofurans (XVa and b), with the expected spectral properties.[1,6]
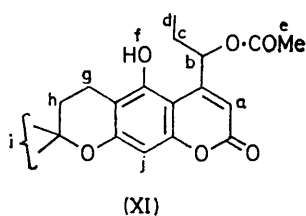
ism by which they act. At the suggestion of Dr. C. B. C. Boyce (Woodstock Agricultural Research Centre). the insecticide (X) was examined as an uncoupler of oxidative phosphorylation. It was active at 0.05 μg ml^{-1}

m/e 430, $C_{24}H_{30}O_7$ (1)

m^* 323.5

m/e 373, $C_{20}H_{21}O_7$ (7)

m^* 350 1

m^* 362.5

m/e 388, $C_{22}H_{28}O_6$ (5)

m/e 370, $C_{22}H_{26}O_5$ (73)

m^* 266

m/e 313, $C_{18}H_{17}O_5$ (54)

m/e 355 (42) $C_{21}H_{23}O_5$

m^* 340.5

m^* 267.5

m^* 211

m/e 327 (23)

m^* 251.8

m/e 299 (15)

m/e 315, $C_{18}H_{19}O_5$ (39)

m/e 257, $C_{14}H_9O_5$ (100)

m^* 204.1

m/e 229, $C_{13}H_9O_4$ (17)

SCHEME Mass spectral fragmentation of insecticidal coumarins

In view of current interest in natural insecticides, it is desirable to know more of the biochemical mechan-
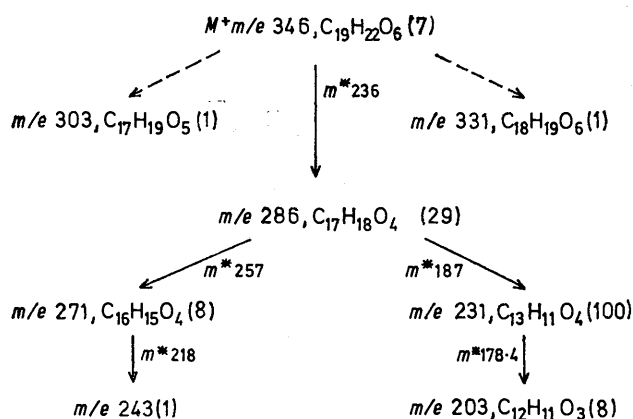
in the test procedure, and, as in the case of mustard beetles, it was substantially more toxic to houseflies (topical and injection), than compounds (I)—(IX). Nonetheless, all compounds of the latter group were also uncouplers at below 0.5 μg ml^{-1} and this property is probably to be found in other members of the group not tested. The 1'-acetoxy-group attached to the 4-alkyl substituent thus appears to be an important factor in the conferment of insecticidal properties. An interesting test of this finding became available at the conclusion of our investigation when there appeared a report of an antibacterial, acetylated coumarin,

(XI)

τ Values

a 3·91 (1H, s)
b 3·54 (1H, dd, J 3 and 8 Hz)
c 8·2—8·6 (2H, m)
d 8·99 (3H, t, J 7 Hz)
e 7·80 (3H, s)
f 2·59 (1H, s, exchanged by D$_2$O)
g 7·60 (2H, t, J 8 Hz)
h 8·30 (2H, t, J 8 Hz)
i 8·75, 8·83 (both 3H, s)
j 3·74 (1H, s)

surangin B (XVI), isolated from *Mammea longifola*
roots.[10] No report of insecticidal activity was made.

$$M^+ m/e\ 346, C_{19}H_{22}O_6\ (7)$$

m*236

$m/e\ 303, C_{17}H_{19}O_5\ (1)$ $m/e\ 331, C_{18}H_{19}O_6\ (1)$

$m/e\ 286, C_{17}H_{18}O_4\ (29)$

m*257   m*187

$m/e\ 271, C_{16}H_{15}O_4\ (8)$ $m/e\ 231, C_{13}H_{11}O_4\ (100)$

m*218   m*178·4

$m/e\ 243\ (1)$ $m/e\ 203, C_{12}H_{11}O_3\ (8)$

In the light of the foregoing, this property would be
expected. Surangin B was examined and found to
uncouple oxidative phosphorylation, to be toxic to

(XII) $R^1 = R^2 = H$
       $R^1 = R^2 = Ac$

(XIII) a; R =
       b; R =

(XIV) a; R =
      b; R =
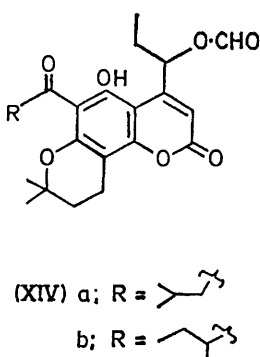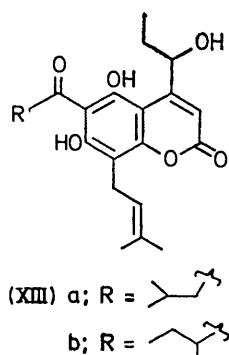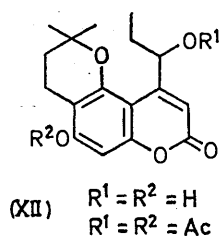
mosquito larvae, and to be more active than our (X),
by both topical and injection methods, to houseflies.

Coumarin types, *e.g.* (V),[1] are distributed in the pulp
and peelings of the fruit of *M. americana*, as well as the
seed. Although used as an edible fruit in the West
Indies, the flesh has long been suspected of being toxic
and connections between this and the uncoupling
activity of the contained coumarins deserve
investigation.

† For details of Supplementary Publications, see Notice to
Authors No. 7 in *J. Chem. Soc.* (*A*), 1970, Issue No. 20.

[10] B. S. Joshi, Y. N. Karnat, T. R. Govindachari, and A. K.
Ganguly, *Tetrahedron*, 1969, **25**, 1453.

(XV) a; R =

     b; R =

(XVI)
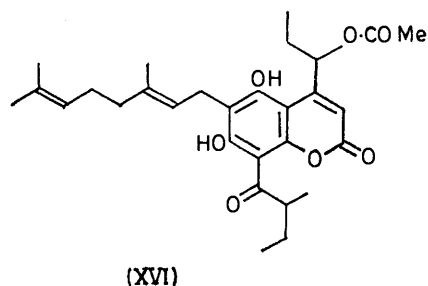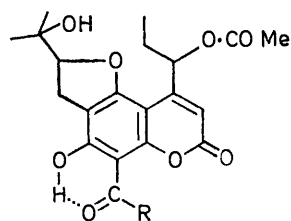
EXPERIMENTAL

For general experimental conditions and meaning of
other symbols, see previous parts. Detailed n.m.r. and
mass spectral data for compounds marked with an asterisk
are deposited with the N.L.L. as Supplementary Publication
No. SUP 20428 (13 pp., 1 microfiche).†

Isolation of Insecticidal Material (X).—Extraction of
the *Mammea americana* L. seeds was described in Part I.[3]
Insecticidal assays, for 5% solutions in acetone against
mustard beetles, were carried out on the chromatography
fractions from the first chromatogram described in Part I.
None of the fractions had insecticidal activity approaching
that of the original light petroleum (b.p. 40—60°) extract.
Continued elution of the chromatogram yielded further
fractions (60—63) [eluant chloroform–ethyl acetate (1 : 3)]
which showed good insecticidal activity (LD_{50} *ca.* 0·01%).
P.l.c. purifications of fractions 60—63, on Merck silica
gel G (methylene chloride as eluant) yielded material, of
high insecticidal activity, intermediate in R_F value between
the yellow crystalline mammea A/B compounds and
the origin. The insecticidal material was further purified
by chromatography on silica gel. Elution with chloroform
yielded material which, when dissolved in the minimum
quantity of carbon tetrachloride and kept in the deep
freeze for several days, yielded white crystals of the in-
secticidal material (2·1 g) m.p. 49—50°, raised to 50—53°
after further chromatography and recrystallisation from
carbon tetrachloride. T.l.c. with a number of different
eluents and plate coatings failed to resolve the material
into more than one spot. The insecticidal material *
gave a violet colour with ethanolic iron(III) chloride
[Found: C, 66·5; H, 6·9%; *M* (mass spec.), 430·1986 ±
22. Calc. for $C_{24}H_{30}O_7$: C, 66·95; H, 7·0%; *M*, 430·1991],
$\nu_{max.}$ 3280, 1725, 1600, and 1560 cm⁻¹, $\nu_{max.}$ (CCl₄) 3350
(unchanged on dilution), 1745, and 1600 cm⁻¹, o.r.d. (*c*
0·04 in EtOH, 25°): $[\phi]_{340}$ −1180°, $[\phi]_{300}$ +753°, $[\phi]_{240}$
−1075°.

Treatment with pyridine–acetic anhydride at room

temperature overnight yielded a white crystalline acetate,* m.p. 20—22° which slowly liquefied at 20° [Found: M (mass spec.), 514·2203 ± 25. Calc. for $C_{28}H_{34}O_9$: M, 514·2203], $\nu_{max.}$ 1775, 1735, 1725, 1610, and 1585 cm^{-1}, $\nu_{max.}$ (film) 1785, 1750, 1710, 1613, 1590, and 1570 cm^{-1}, $\lambda_{max.}$ 218, 284, and 321 nm (log ε 4·83, 4·44, and 4·21).

The dimethyl ether * had m.p. 88—90° and like the diacetate give no colour with iron(III) chloride solution [Found: M (mass spec.), 458·2304 ± 23. Calc. for $C_{26}H_{34}O_7$: M, 458·2304], $\nu_{max.}$ (CCl$_4$) 1745, 1710, 1615, and 1580 cm^{-1}, $\nu_{max.}$ (mull) 1745, 1710, 1615, and 1585 cm^{-1}, $\lambda_{max.}$ 218, 295, and 316 nm (log ε 4·98, 4·09, and 3·97).

Action of 98% Formic Acid on the Insecticidal Material (X).—Insecticidal material (X) (100 mg) was dissolved in 98% formic acid (2 ml) and the solution was heated on a boiling water-bath for 2 h. The solution was poured on ice to give the white crystalline chroman * (XI) (50 mg), m.p. 247—249° (from ethanol) [Found: C, 65·9; H, 6·25%; M (mass spec.), 346·1409 ± 15. Calc. for $C_{19}H_{22}O_6$: C, 65·9; H, 6·4%; M, 346·1416]. The chroman gave a positive Gibbs test, performed under spectrophotometric control (see Part I [3]) [$\lambda_{max.}$ 675 nm (log ε 4·36)]; $\nu_{max.}$ 3350, 1730, 1630, and 1570 cm^{-1}, $\nu_{max.}$ (CCl$_4$) 3260, 1740, 1645, and 1610 cm^{-1}, $\nu_{max.}$ (mull) 3380, 1740, 1695, 1630, 1610, and 1570 cm^{-1}, $\lambda_{max.}$ 250, 260, and 329 nm (log ε 3·73, 3·73, 4·19) (no change in ethanolic 0·01N-HCl), $\lambda_{max.}$ (ethanolic 0·01N-KOH) 277, 337, and 404 nm (log ε 4·10, 4·01, and 3·86), o.r.d. (c 0·025 in EtOH, 25°): [ϕ]$_{345}$ +800°, [ϕ]$_{280}$ −2650°, [ϕ]$_{250}$ −4860°. For mass spectral breakdown see text.

Heating the insecticidal material with trifluoroacetic acid yielded the same chroman (XI). The chroman on treatment with pyridine–acetic anhydride gave a non-crystalline acetate * [Found: C, 64·8; H, 6·1%; M (mass spec.), 388·1522 ± 19. Calc. for $C_{21}H_{24}O_7$: C, 64·95; H, 6·25%; M, 388·1522], $\nu_{max.}$ (film) 1780, 1735, 1625, and 1560 cm^{-1}, $\nu_{max.}$ (CCl$_4$) 1785, 1735, and 1625 cm^{-1}, $\lambda_{max.}$ 248, 259, and 330 nm (log ε 3·63, 3·53, and 4·00).

Action of 75% Sulphuric Acid on Insecticidal Material.— Insecticidal material (X) (160 mg) in 75% aqueous sulphuric acid (5 ml) was stirred at room temperature for 2 days. The solution was poured into water and 3,4-*dihydro-5-hydroxy*-10-(1-*hydroxypropyl*)-2,2-*dimethyl*-2H,8H-*benzo*-[1,2-b:3,4-b′]*dipyran-8-one* * (XIIa) (20 mg) was collected, m.p. 242—244° (decomp.) (from acetone–hexane) [Found: M (mass spec.), 304·1311 ± 15. $C_{17}H_{20}O_5$ requires M, 304·1311], $\nu_{max.}$ (mull) 3310, 3140, 1705, and 1610 cm^{-1}, $\lambda_{max.}$ 249, 258, and 327 nm (log ε 3·84, 3·82, and 4·28) (no change in ethanolic 0·01N-HCl), $\lambda_{max.}$ (ethanolic 0·01N-KOH) 239, 272, and 382 nm (log ε 4·17, 3·80, and 4·26). The compound gave a negative Gibbs test under spectrophotometric control.

Treatment with pyridine–acetic anhydride gave a non-crystalline diacetate * (XIIb) [Found: M (mass spec.), 388·1522 ± 19. Calc. for $C_{21}H_{24}O_7$: M, 388·1522], $\nu_{max.}$ (film) 1775, 1740, and 1620 cm^{-1}, $\lambda_{max.}$ 273 nm (log ε 4·72), o.r.d. (c 0·06 in EtOH): [ϕ]$_{270}$ −1850°, [ϕ]$_{255}$ −550° [ϕ]$_{240}$ −1100°.

Isomerisation of the Insecticidal Material (X).—The insecticidal material (140 mg) and methanolic 5% potassium hydroxide (5 ml) were kept at 20° overnight, poured into water (20 ml), and acidified with 4N-hydrochloric acid. Extraction with ether gave a yellow gum (100 mg) which failed to crystallise. The coumarin mixture * (XIIIa and b) formed was a yellow glass which softened

and became liquid over a range (63—73°), and gave a green colour with ethanolic iron(III) chloride solution [Found: M (mass spec.), 388·1886 ± 18. Calc. for $C_{22}H_{28}O_6$: M, 388·1886], $\nu_{max.}$ (film) 3330, 1710, 1605, and 1585 cm^{-1}, $\nu_{max.}$ 3200, 1715, 1605, and 1580 cm^{-1}, $\nu_{max.}$ (CCl$_4$) 3300, 1705, and 1600 cm^{-1}, $\lambda_{max.}$ 280 and 330 nm (log ε 4·31 and 3·92), $\lambda_{max.}$ (ethanolic 0·01N-KOH) 234, 303, and 400 nm (4·20, 4·21, and 4·00).

Treatment with pyridine–acetic anhydride gave a mixture of non-crystalline triacetates * [Found: M (mass spec.), 514·2203 ± 26. Calc. for $C_{28}H_{34}O_9$: M, 514·2203], $\nu_{max.}$ 1780, 1735, 1620, and 1590 cm^{-1}, $\nu_{max.}$ (film) 1783, 1745, 1710, 1620, and 1595 cm^{-1}, $\lambda_{max.}$ 252, 280, and 314 nm (log ε 3·92, 3·94, and 3·61).

Action of 98% Formic Acid on Coumarins (XIIIa and b).— The coumarins (150 mg) were treated with 98% formic acid (10 ml) as above to give the chromans * (XIVa and b) (70 mg), m.p. 163—167° (from chloroform–hexane) [Found: M (mass spec.), 416·1835 ± 21. Calc. for $C_{23}H_{28}O_7$: M, 416·1835], $\nu_{max.}$ 1730, 1618, and 1595 cm^{-1}, $\nu_{max.}$ (mull) 3320, 1725, 1695, 1620, and 1600 cm^{-1}, $\nu_{max.}$ (film) 3400, 1730, 1615, and 1590 cm^{-1}, $\lambda_{max.}$ 282 and 325 nm (log ε 4·31 and 3·96), $\lambda_{max.}$ ethanolic 0·01N-KOH) 235infl. 285, 310, and 287 nm (log ε 4·12, 3·89, 3·92, and 3·77).

The chroman mixture (XIVa and b) gave non-crystalline acetates * [Found: M (mass spec.), 458·1941 ± 23. Calc. for $C_{25}H_{30}O_8$: M, 458·1941], $\nu_{max.}$ 1780, 1720, and 1590 cm^{-1}, $\nu_{max.}$ (film) 1785, 1730, and 1595 cm^{-1}, $\lambda_{max.}$ 260infl, 285, and 325 nm (log ε 3·92, 4·08, and 4·01).

Reactions of m-*Chloroperbenzoic Acid with Insecticidal Material* (X).—A solution of the insecticidal material (150 mg) and m-chloroperbenzoic acid (100 mg) in dry dioxan (10 ml) was stirred at 20° for 3 h, then diluted with ether (20 ml) and washed with aqueous 10% sodium thiosulphite (5 ml), aqueous 5% sodium hydrogen carbonate solution (2 × 5 ml), and saturated sodium chloride (5 ml). After drying and removal of the ether a yellow gum * (XVa and b) (100 mg) was obtained, which gave only one spot on t.l.c. [Found: M (mass spec.), 446·1944 ± 22. Calc. for $C_{24}H_{30}O_8$: M, 466·1941], $\nu_{max.}$ 1730, 1630, and 1610 cm^{-1}, $\nu_{max.}$ (film) 3440, 1730, 1630, and 1610 cm^{-1}, $\lambda_{max.}$ 257, 267, and 295 nm (log ε 4·08, 4·06, and 4·00), $\lambda_{max.}$ (ethanolic 0·01N-KOH) 250infl, 315, and 370infl nm (log ε 4·17, 3·86, and 3·73.)

Treatment with pyridine–acetic anhydride at room temperature yielded non-crystalline monoacetates * (50 mg) [Found: M (mass spec.), 488·2040 ± 24. Calc. for $C_{26}H_{32}O_9$: M, 488·2046], $\nu_{max.}$ 1775, 1740, 1630, 1610, and 1590 cm^{-1}, $\nu_{max.}$ (film) 3500, 1775, 1740, 1625, 1605, and 1590 cm^{-1}, $\lambda_{max.}$ 227, 258, and 296 nm (log ε 4·18, 3·97, and 4·07).

Ozonolysis of the Insecticide (X).—Ozone was passed through a solution of the insecticidal material (120 mg) in glacial acetic acid (5 ml). The solution was stirred with iron(II) sulphate solution for 1 h and the clear liquid was decanted off. Aspiration into 2,4-dinitrophenylhydrazine reagent gave the 2,4-dinitrophenylhydrazone of acetone (45 mg), m.p. 121—122° (mixed m.p. and i.r. comparison).

Identification of Acyl Substituents of the Insecticidal Material (X).—The insecticidal material (200 mg) was dissolved in 0·2N-sodium hydroxide (5 ml) and cooled to 0°, and hydrogen peroxide (30% w/v; 3 ml) was added. The mixture was kept at 0° for 3 h, then a 2 ml portion of the solution was pipetted on to a 1 cm diam. column of sodium hydrogen sulphate (15 g). The column was

eluted with ether (70 ml). The eluate was dried, and evaporated to 2 ml. A second 2 ml portion of the solution was treated similarly and the two solutions were combined. A 25% solution of sulphuric acid in propan-2-ol (0·1 ml) was added. Residual ether was removed and the solution was sealed in a heavy-walled glass ampoule and heated in a boiling water bath for 2·5 h. The ampoule was cooled and opened, water (0·2 ml) and ether (0·2 ml) were added, and the mixture was shaken. The upper layer was removed and portions (1 μl) were examined by g.l.c.

The g.l.c. was carried out on a glass column (5% UCON on Chromosorb W, H.M.D.S., as the stationary phase) at 45°. From the insecticide (X), the isopropyl esters of 3-methylbutyric and 2-methylbutyric acids were obtained in the ratio 1·0 : 1·4; a trace of isopropyl n-butyrate (<0·1) was detected. Identification was carried out by comparison of retention times with those of authentic specimens and also by co-chromatography.

A contaminated sample of Mammea B/BB was degraded in a similar manner. G.l.c. analysis showed the presence of isopropyl 2-methylbutyrate and 3-methylbutyrate in the ratio 10 : 1. Isopropyl n-butyrate was also identified. Degradation of synthetic 5,7-dihydroxy-6-(2-methylbutyryl)-4-n-propylcoumarin gave only isopropyl 2-methylbutyrate and n-butyrate, whereas degradation of 5,7-dihydroxy-4-n-propylcoumarin gave only n-butyric acid, originating from the 4-side chain.