Constituents of *Mammea americana* L. VIII: Novel Structural Variations on the Mammein Theme and Antitumor Activity of Mammein and Related Coumarin and Phloroglucinol Derivatives

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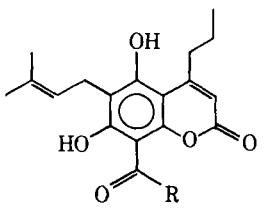
Abstract □ Mammein (4-*n*-propyl-5,7-dihydroxy-6-isopentenyl-8-isovalerylcoumarin) was shown to be accompanied by two difficultly separable congeners: the 8-α-methylbutyryl isomer (neomammein) and the 8-*n*-butyryl homolog (normammein). In addition, new analogs of these compounds in which the 6-isopentenyl groups have undergone oxidative cyclization with the 5-hydroxyl groups were isolated and identified by spectroscopic means. Each of these substances was also obtained by peracid oxidation of its noncyclized relatives. Significant antitumor activity against Sarcoma 180 grown in stationary cell culture was exhibited by mammein and certain of its companions, as well as by various degradation and synthetic intermediates.

Keyphrases □ *Mammea americana* L.—isolation, identification of constituents, antitumor activity □ Mammein, related coumarin and phloroglucinol derivatives—structure identification, antitumor activity □ Medicinal plants—constituents of *Mammea americana* L., antitumor activity □ Antitumor activity—mammein, related coumarin and phloroglucinol derivatives

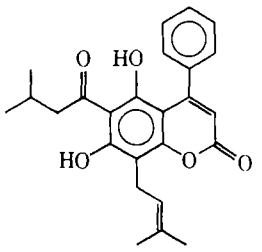
Seed extracts of *Mammea americana* L. (family Guttiferae) have proven to be a rich source of oxygen heterocyclic compounds of the coumarin (1–5) and xanthone (5–7) classes. The extensive efforts of Djerassi and his collaborators (1, 8, 9), which culminated in Structure I for mammein and revealed in detail the variegated chemistry of this polyfunctional molecule, established a precedent which greatly facilitated access to Structures II for mammeisin (2) and III for mammeigin (3). Thus, Structure II was secured relatively quickly on the basis of experiments conducted in the mammein mold, while Structure III, although based principally on its NMR spectrum, was easily verified by a chemical interconversion with II. In 1965, the authors learned[1] that samples of mammein (I) emanating from this laboratory and elsewhere were, in fact, mixtures of I, the major constituent, with its isomer IV and its desmethyl analog V. This discovery was made possible by applying the techniques of NMR, mass spectroscopy, and TLC, none of which was utilized during the original studies on I. In light of these revelations[2], an examination was begun of a variety of available mammein samples[3]; our findings were in agreement with those of Crombie and his collaborators. In addition, during the reisolation of I, IV, and V from mamey oil, a number of new substances were encountered, one of which was assigned Structure VI (accompanied by smaller amounts of VII and VIII) on the basis of arguments presented below. Finally, the results of a preliminary biological assay of these and allied substances are reported.

DISCUSSION

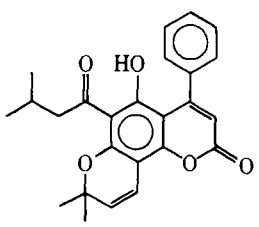
Mammein Mixture—In view of the close structural relationship of neomammein (IV) and normammein (V) to mammein (I), it is not surprising that the presence of IV and V went unsuspected for so many years. Even when pure, the melting points of these substances are within a few degrees of one another and, of course, they do not depress on admixture. Furthermore, their IR spectra, which are exceptionally rich in detail, are virtually identical throughout the entire region, with the exception of minor variations near 1150 cm.$^{-1}$. It is ironic, in retrospect, to recall that these minor differences in the spectra observed[4] for various chromatographic fractions of mammein encountered during the original studies (1) and ascribed to the presence of "impurities" were, in fact, a real, albeit subtle, indication of the mixture in hand. Especially ironic is the fact that one of these original samples[5], which was not employed in the structural studies, has now been shown to be pure neomammein (IV).



I: R = isobutyl, mammein (mammea B/BA)
IV: R = *sec*-butyl, neomammein (mammea B/BB)
V: R = *n*-propyl, normammein (mammea B/BC)



II: mammeisin (mammea A/AA)



III: mammeigin (mammea A/A cyclo D)

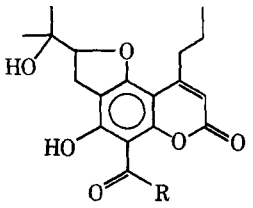
[1] From Professor L. Crombie and Dr. D. E. Games.

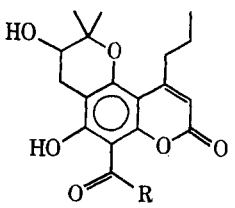
[2] The authors are indebted to Professor Crombie and Dr. Games for experimental detail, including copies of spectra and comparison samples. They also acknowledge the receipt of prepublication copies of References 4, 5, 10, and 11.

[3] The finding of a large number of closely related coumarins in mamey oil has generated a nomenclature problem for which a solution was advanced by Crombie (4) in the form of a letter code. These designations are included in parentheses under the structural formulas.

[4] R. A. Finnegan, unpublished observations, 1958.

[5] The authors thank Professor Djerassi for the gift of a large number of samples accumulated in his laboratory during the original studies.





VI: R = *sec*-butyl
VII: R = isobutyl
VIII: R = *n*-propyl
(mammea L-1)

IX

Compounds I, IV, and V can be obtained in reasonable purity by repeated chromatography on alumina or silica gel columns. The course of the separation is readily monitored by TLC and NMR measurements, and the substances obtained were found by direct comparison to be identical with those isolated in Crombie's[2] laboratory. A detailed discussion of the NMR (as well as mass) spectra of these compounds was published (10) and need not be dealt with here.

Cycloneomammein (VI)—In the course of chromatographic separations of mammein and its close relatives, a new crystalline substance, m.p. 130–131°, was encountered. It showed IR and UV spectral properties similar to other phenolic *Mammea* coumarins bearing the acyl substituent at C-8 (8–10). Microanalytical data accorded with the formula $C_{22}H_{28}O_6$, and this was verified by the observation of a parent peak in its mass spectrum at *m/e* 388. The compound was optically active, showed a positive ferric chloride test, and readily formed a monoacetate derivative, m.p. 134–135.5°. Although this derivative no longer possessed phenolic properties, hydroxyl absorption was still evident in the IR, indicating the additional presence of an aliphatic hydroxyl group. Examination of the NMR spectrum clearly showed its relationship to its companions and allowed a detailed structure to be assigned as shown in VI.

The low field position of the phenolic proton (exchangeable in D_2O) at -3.90τ indicates that it is hydrogen bonded to the carbonyl group at C-8. This rules out structures in which the rings are linearly fused, because the phenolic hydroxyl would then be situated at C-5, unable to chelate with the keto group, and thus would absorb at much higher field. A second hydroxyl line (exchangeable in D_2O) appears at 7.70τ and is assigned to the aliphatic hydroxyl function. A singlet at 4.12τ is typical for the C-3 hydrogen of the coumarin nucleus and, since it is not coupled, shows that C-4 bears a substituent other than hydrogen. The substituents at C-4 and C-8 were seen, by comparison, to be identical with the correspondingly placed substituents in neomammein (IV). It was apparent, however, that the isopentenyl side chain present in I, IV, and V had been replaced by a new functionality, giving rise to two methyl singlets (8.59 and 8.71τ), a single-proton triplet at 5.16τ (J = 9 Hz.), and a two-proton doublet centered at 6.83τ (J = 9 Hz.). While these absorptions could, in principle, be satisfied by either the hydroxyisopropyl dihydrofurano moiety in VI or by the hydroxy dimethylchromano grouping as illustrated in IX, the choice was easily made in favor of VI by comparison of the relevant chemical shifts with those reported in the literature (12–15) for a number of suitable model compounds. Furthermore, the presence in the mass spectrum of a peak at *m/e* 59 (9%) [$(CH_3)_2C^+OH$] is more compatible with Structure VI than with IX (16, 17). The NMR spectrum of an oily *diacetate* (which could not, however, be obtained in analytical purity), provided additional support for Structure VI in that the downfield shift of the methine proton was 0.27 p.p.m., only a small fraction of that expected for the corresponding shift in IX.

Although the substance to which Structure VI was assigned gave only one spot on TLC analysis, it is not homogeneous. The presence of its lower homolog VIII was indicated by a peak in the mass spectrum at *m/e* 374, 14 units lower than the parent ion of VI and very unlikely to be derived therefrom. In some samples, the abundance of the 374 ion was nearly the same as that of the 388 ion. This does not necessarily reflect the composition of the sample, however. Since the base peak is formed by the loss of the R group, one might expect the parent ion of VI to be less stable than that of VIII (easier loss of *sec*-butyl radical than *n*-propyl radical). The presence of VII (and VIII) can be ascertained by careful examination of the NMR spectrum, particularly the methyl region and the region near 7τ (CH_2 next to CO). In these samples, VI was clearly the major constituent as judged by the prominence of the doublet at 8.78τ [CH_3—C(—)HCO—] and the multiplet centered at 6.20τ [—C(—)HCO—], which are distinctive for this particular side chain.

Epoxidation of I, IV, and V—Treatment of mammein (I) (containing IV and V) with monoperphthalic acid provided, in good yield, the cyclized product VII. This product was identical with the material (VI, VII, and VIII) previously isolated, except for the distribution of C-8 substituents. In addition, the separated constituents, I, IV, and V, were *individually* oxidized with peracid in order to provide homogeneous samples of VII, VI, and VIII, respectively. These transformations, of course, served to provide complete verification of the structures assigned to VI and its companions. Although the epoxide of mammein diacetate could be prepared easily, we were unable, at first, to prepare the epoxide of I itself, which is the presumed intermediate between I and VII. Subsequent experiments, however, were successful (18).

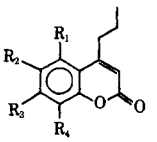
Finally, the independent isolation of a similar mixture of Compounds VI, VII, and VIII by Crombie and his coworkers led to joint preliminary communication of these results (19, 20).

Cytotoxicity—The cytotoxic effect of mamey oil and a number of its constituents against Sarcoma 180 tumor cells grown in stationary cell culture was determined. These results, expressed as the concentration in micrograms per milliliter required to produce 50% inhibition of growth, ID_{50}, are assembled in Table I. Values less than 5 are taken to indicate significant activity. Table II lists similar

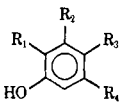
Table I—Activity of Mammein and Related Coumarins against Sarcoma 180 Tumor Cells

Substance	ID_{50}, mcg./ml.
Mamey oil (dewaxed)	0.3
Mammein (I, containing IV and V)	0.07
Neomammein (IV)	<0.1
Mammein (I, containing some V)	<0.03
Mammein diacetate (containing diacetates of IV and V)	0.04
Dihydromammein (I, with double bond in side chain reduced, containing some dihydro-IV and dihydro-V)	0.08
Cycloneomammein (VI, containing some VII and VIII)	0% inhibition at 100 mcg./ml.
Mammeisin (II)	<0.1
Dihydromammeisin (II, with double bond in side chain reduced)	<0.1
C_{22}-Aldehyde from mammeisin (aldehyde derived from II by ozonolysis of the double bond) (2)	1.8
Cyclomammeisin (dihydrofurano compound derived from II by peracid oxidation) (18)	80
Anhydrocyclomammeisin (benzofuran obtained by dehydration of cyclomammeisin) (18)	56

Table II—Activity of Some Coumarins and Phloroglucinols against Sarcoma 180 Tumor Cells



R_1	R_2	R_3	R_4	ID_{50}, mcg./ml.
OH	H	OH	H	34
OCH_3	H	OCH_3	H	20
OCH_3	H	OCH_3	Isopentyl	3.7
OH	Isopentyl	OH	H	<0.1
OH	Isovaleryl	OH	H	2.7



Isopentyl	OH	H	OH	42
Isovaleryl	OH	H	OH	19
Isovaleryl	OCH_3	H	OCH_3	25
Isovaleryl	OH	Isopentyl	OH	2.1

data for several related coumarins and phloroglucinols which were synthesized in the course of the original structure determination of I (9).

It is clear from Table I that the *Mammea* coumarins and their simple relatives, with the notable exception of the cyclo derivatives, all possess considerable activity in this assay, and it is suggestive that some of the less complicated analogs (Table II) retain some of this activity. Additional biological assays on several of these compounds is certainly in order and further synthetic studies in this area may well prove rewarding.

EXPERIMENTAL[6]

Isolation of Mammein Mixture from Mamey Oil—The preparation of mamey oil has been described before (3). In this work, various samples of oil were chromatographed quickly through a column of alumina (Merck, acid washed, deactivated with 5% water). For example, 160 g. oil dissolved in 160 ml. hexane would be placed on a column (840 × 70 mm.) containing 3225 g. alumina packed in hexane. Fractions eluted with benzene or benzene–chloroform mixtures provided, after evaporation, crystalline residues which were triturated with hexane and filtered. After being washed with hexane and dried, the resulting solids were found by TLC analysis to contain about equal proportions of I, IV, and V. In several experiments, a total of 1160 g. of mamey oil provided 56.6 g. of almost white crystals. A single recrystallization from ether–hexane furnished 48 g. of white needles, m.p. 124–129°. Comparison of the IR and UV spectral data with those reported for mammein showed them to be identical. TLC on silica gel G plates eluted with chloroform (or methylene chloride) containing 0.2–2.0% acetic acid gave a clear separation of the individual components I, IV, and V. These components were positively identified by the simultaneous application to the plates of authentic samples[2]. The order of decreasing mobility is IV > I > V. A number of other TLC systems with different adsorbent and solvent combinations were investigated; however, no separation was achieved. The majority gave only elongated spots without separating the individual components. The NMR spectrum was also examined and the presence of I, IV, and V was verified by comparison with the spectrum of each component.

Other samples of the mammein mixture were obtained by direct separation of a crystalline residue from a number of mamey oil samples which had been standing for several years. The residues were obtained by decantation and, after being thoroughly washed with hexane and dried, they were shown by TLC and NMR to be mixtures of I, IV, and V.

A total of 16 crystalline "mammein" samples of different origin were compared by melting point, TLC, NMR, and IR with authentic specimens of the components. These samples were obtained by either the chromatography or decantation method already described, or they were available from previous sample collections. Most of these were shown to be mixtures of I, IV, and V, although two were two-component mixtures (IV + I and I + V) and one was pure IV. The melting points of the 16 samples ranged between 110 and 132°, and their IR spectra showed no "significant" differences.

Chromatographic Separation of Individual Components of Mammein Mixture—The crystalline mixture, m.p. 124–129° (1 g.), was preadsorbed on 8 g. silica gel and placed on a column (350 × 20 mm.) containing 55 g. silica gel packed in hexane. Elution was carried out (500-ml. fractions) with hexane, benzene, chloroform, and mixtures of these solvents. The residues from each fraction were examined by TLC and treated as described in the previous experiment. Mixtures of chloroform and hexane were used for the final recrystallizations. A period of 4 days was required to elute the fractions containing the "mammein" components. From Fractions 16–20 (2:1 hexane–benzene), there was obtained 194 mg. residue which was pure neomammein (IV) as determined by its TLC behavior. Recrystallization provided 99 mg. with m.p. 121–122° [lit. (10) m.p. 122°]. Fractions 21 and 22 provided an additional 105 mg. IV (58 mg., m.p. 122–123°, after recrystallization) which showed the presence of traces of I by TLC. Fractions 23–26 gave 152 mg. of a mixture of I and IV; Fractions 27–29 (87 mg., 50 mg. after recrystallization, m.p. 126–127°) afforded nearly pure I contaminated by a small amount of IV; and Fraction 30 (1:1 hexane–benzene) gave pure mammein (I) (84 mg.) which had m.p. 127° [lit. (10) m.p. 127°] after recrystallization (24 mg.). Fractions 31–33 contained a mixture (107 mg.) of I and V (54 mg., m.p. 131–132°, after recrystallization), and Fractions 34–36 (benzene) afforded pure V (199 mg., 87 mg. after recrystallization, m.p. 130–132°) [lit. (10) m.p. 132–133°].

The pure samples of I, IV, and V obtained in this way had spectroscopic properties identical with those of the corresponding samples isolated in Crombie's laboratory[2]. In addition, it was observed that neomammein (IV) had the following optical activity: $[\alpha]^{25.5°}_{589,578,546,436}$ −2.93, −3.41, −4.14, −14.5 (c, 0.0434 g./ml., chloroform).

Isolation of Cycloneomammein (VI)—In a larger scale experiment, 10.7 g. of the crystalline mammein mixture was preadsorbed on 50 g. silica gel and placed on a column (600 × 40 mm.) of 400 g. silica gel packed in hexane. Fractions of 1000 ml. were collected, and the residues were examined by TLC. The first 43 fractions (mainly 2:1 hexane–benzene) gave only small amounts (total 811 mg.) of oily residues and were not further investigated. Fractions 44–79 (2:1 hexane–benzene to 1:3 hexane–benzene) contained I, IV, and V and were treated as described in the previous experiment. The following were obtained: 960 mg. IV, 1.33 g. of a mixture of I and IV, 427 mg. of I, and 424 mg. of a mixture of I and V.

Fractions 80–113, eluted with benzene and 4:1 benzene–chloroform, afforded 1.75 g. of a substance known as Compound L-2 (18).

Fractions 114–125 (4:1 benzene–chloroform) provided, after a single recrystallization, 1.24 g. of cycloneomammein, m.p. 114–128°. Decolorization with charcoal and four recrystallizations from ether gave colorless platelets, m.p. 130–131°; ν^{KBr}_{max}: 3460, 2959, 1715, 1634, 1603, 1433, 1408, 1389, 1361, 1203, 1130, 1114, 948, and 895 cm.$^{-1}$; $\lambda^{95\%\ ethanol-0.01\ N\ HCl}_{max}$: 222.5, 231 (sh), and 300 nm., log ϵ: 4.39, 4.26, and 4.44; $\lambda^{95\%\ ethanol-0.01\ N\ KOH}_{max}$: 215, 242.5, 315 (sh), and 380 nm.; log ϵ: 4.61, 4.44, 3.79, and 4.33; $[\alpha]^{29°}_{589,578,546,436}$ −1.48, −1.46, −1.78, −5.44 (c, 0.050 g./ml., chloroform).

Anal.—Calc. for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27; O, 24.71. Found: C, 68.12; H, 7.41; O, 24.78.

This substance gave a purple color with ferric chloride[7].

Monoacetate of VI—A solution of VI (200 mg.) in pyridine (1.5 ml.) and acetic anhydride (1.5 ml.) was stirred for 24 hr. at room temperature. The reaction mixture was poured into ice water (30 ml.), and the mixture was allowed to stand for several hours. The solid material was then separated by filtration, washed with water, and dried. Recrystallization from chloroform–hexane gave a crystalline white solid (82 mg.), m.p. 127–130°. Four recrystallizations from aqueous ethanol raised the melting point to 136–137°; ν^{KBr}_{max}: 3559, 2959, 1783, 1742, 1692, 1626, 1600, 1439, 1192, 1124, and 1100 cm.$^{-1}$. The compound gave a negative ferric chloride test.

Anal.—Calc. for $C_{24}H_{30}O_7$: C, 66.96; H, 7.02. Found: C, 66.94; H, 6.93.

Reaction of Mammein Mixture with Monoperphthalic Acid—A solution of "mammein" (see first experiment) (500 mg., 1.35 mmoles) in ether (25 ml.) was added dropwise to an ice-cooled solution of monoperphthalic acid (450 mg., 2.47 mmoles) in ether (3 ml.). The reaction mixture was stirred for 6 hr. at 0° and then allowed to stand for 12 hr. at −15°. TLC still showed unreacted starting material; therefore, an additional amount of peracid (150 mg., 1.0 mmole) was added and the mixture was stirred for another 4 hr. at 0°. Complete consumption of starting material was shown

[6] All melting points were determined with a Fisher-Johns block and are uncorrected. IR spectra were taken on a Perkin-Elmer model 237 spectrophotometer. Only characteristic and/or strong absorptions (50% of strongest band) are reported. UV and visible spectra were obtained with a Beckman DB-G spectrophotometer. The UV spectra of the phenolic compounds were determined in 0.01 *N* alcoholic hydrochloric acid and alcoholic hydroxide solutions, which were prepared by adjusting the alcoholic (95%) solutions of the compounds to 0.01 *N* by the addition of concentrated hydrochloric acid or 10 *N* NaOH, respectively. NMR spectra were determined with a Varian A-60 instrument. All solvents used for chromatography, reaction media, or recrystallizations were reagent grade or purified by distillation. Column chromatography was carried out with acid-washed alumina from Merck, neutral alumina from Woelm, and silica gel from British Drug Houses. TLC was done with silica gel G, and spots were detected by exposing the plates to iodine vapor. Solutions were concentrated by rotary evaporation at 60–70° and reduced pressure (aspirator). Microanalyses were performed by the Mikroanalytisches Laboratorium im Max Planck Institut für Kohlenforschung, Mühlheim, Germany.

[7] See the text for a discussion of the fact that VI as obtained in this way is admixed with VII and VIII.

by TLC. Insoluble phthalic acid was removed by filtration; the filtrate was then washed with saturated aqueous sodium bicarbonate solution (4 × 20 ml.), water (4 × 20 ml.), and saturated aqueous sodium chloride solution (2 × 20 ml.) and dried with anhydrous magnesium sulfate. Evaporation of the solvent gave an almost white crystalline residue (347 mg.). This solid was triturated with ether, the ether was removed by decantation, and the remaining solid residue was dried to give 303 mg. (58%) of white crystals, m.p. 115–125°. Four recrystallizations from ether raised the melting point to 127–128°. This product, a mixture of VI, VII, and VIII, had IR, UV, and NMR spectral data nearly indistinguishable from those of the "natural" material isolated by chromatography of crude mammein mixture.

Cycloneomammein (VI) by Peracid Treatment of Neomammein (IV)—A solution of neomammein (IV) (385 mg., 1.0 mmole) in chloroform (2 ml.) and ether (8 ml.) was added dropwise to a stirred solution of monoperphthalic acid (405 mg.) in ether (6 ml.) at 0°. The reaction mixture was kept for 12 hr. at 0° and then for 12 hr. at −15°. The phthalic acid was separated by filtration and washed with ether. The filtrate and ether washings were combined; they were then washed with water (2 × 10 ml.), saturated aqueous sodium bicarbonate solution (10 × 10 ml.), water (3 × 15 ml.), and saturated sodium chloride solution (2 × 15 ml.) and dried with anhydrous magnesium sulfate. Evaporation afforded a crystalline residue, m.p. 116–120°, which was recrystallized from ether to give colorless platelets of VI, m.p. 122–123°, 310 mg. (77%). Two additional recrystallizations raised the melting point to 124–125°; ν_{max}^{KBr}: 3460, 2950, 1715, 1634, 1603, 1408, 1387, 1203, 1133, 1122, and 894 cm.$^{-1}$; $\lambda_{max}^{95\%\ ethanol-0.01\ N\ HCl}$: 220, 232 (sh), and 297 nm.; log ε: 4.41, 4.26, and 4.46; $\lambda_{max}^{95\%\ ethanol-0.01\ N\ NaOH}$: 218, 240, 320 (sh), and 375 nm.; log ε: 4.21, 4.43, 3.85, and 4.32; NMR ($CDCl_3$–tetramethylsilane): τ −4.10 (1H, s), 4.06 (1H, s), 5.12 (1H, t, J = 9 Hz.), 6.19 (1H, m), 6.82 (2H, d, J = 9 Hz.), 7.13 (2H, t, J = 7 Hz.), 7.30 (1H, s), 8.0–8.7 (4H, m), 8.55 (3H, s), 8.70 (3H, s), 8.78 (3H, d, J = 7 Hz.), 8.97 (3H, t, J = 7 Hz.), and 9.05 (3H, t, J = 7 Hz.).

Anal.—Calc. for $C_{21}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 68.14; H, 7.49.

Cyclomammein (VII) by Peracid Treatment of Mammein (I)—A solution of mammein (I) (204 mg., 0.55 mmole) in chloroform (1 ml.) and ether (4 ml.) was added dropwise to a stirred solution of monoperphthalic acid (270 mg.) in ether (6 ml.) at 0°. The reaction mixture was stirred for 10 min. at 0° and then kept for 10 hr. at −15°. Since traces of starting material were found by TLC, another portion of peracid (135 mg.) in ether (1 ml.) was added to the reaction mixture and stirring was continued for 1 hr. at room temperature. The reaction mixture was then worked up in the same manner as described in the preceding experiment to give a slightly brown solid, 211 mg. Recrystallization from ether afforded tan platelets, m.p. 127–128°, 139 mg. (65%). Two additional recrystallizations from ether gave colorless platelets of VII, m.p. 128.5–129.5° (121 mg.); ν_{max}^{KBr}: 3448, 2950, 1715, 1634, 1600, 1431, 1404, 1387, 1353, 1200, 1134, 1117, and 894 cm.$^{-1}$; $\lambda_{max}^{95\%\ ethanol-0.01\ N\ HCl}$: 221, 231 (sh), and 295 nm.; log ε: 4.39, 4.25, and 4.44; $\lambda_{max}^{95\%\ ethanol-0.01\ N\ NaOH}$: 241, 320 (sh), and 377 nm.; log ε: 4.39, 3.80, and 4.32; NMR ($CDCl_3$–tetramethylsilane): τ −4.10 (1H, s), 4.07 (1H, s), 5.12 (1H, t, J = 9 Hz.), 6.81 (2H, d, J = 9 Hz.), 6.93 (2H, d, J = 7 Hz.), 7.13 (2H, t, J = 7 Hz.), 7.30 (1H, s), 7.74 (1H, m), 8.2–8.6 (2H, m), 8.55 (3H, s), 8.70 (3H, s), 8.97 (3H, t, J = 7 Hz.), and 8.99 (6H, d, J = 6.5 Hz.).

Anal.—Calc. for $C_{21}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 67.83; H, 7.45.

Cyclonormammein (VIII) by Peracid Treatment of Normammein (V)—A solution of normammein (V) (246 mg., 0.686 mmole) in chloroform (1 ml.) and ether (4 ml.) was added dropwise to a stirred solution of monoperphthalic acid (405 mg.) in ether (7 ml.) at 0°. The reaction mixture was kept for 12 hr. at 0° and then for 12 hr. at −15° before it was worked up in the same manner as in the preceding two experiments. Recrystallization of the crude product from ether gave tan platelets of VIII, m.p. 126–128° (203 mg., 79%). Four additional recrystallizations, including one treatment with charcoal, gave colorless platelets, m.p. 130–131°; ν_{max}^{KBr}: 3448, 2950, 1712, 1631, 1595, 1431, 1403, 1385, 1353, 1196, 1139, 1130, 1112, and 894 cm.$^{-1}$; $\lambda_{max}^{95\%\ ethanol-0.01\ N\ HCl}$: 223, 236 (sh), and 295 nm.; log ε: 4.37, 4.22, and 4.42; $\lambda_{max}^{95\%\ ethanol-0.01\ N\ NaOH}$: 239, 320, (sh), and 375 nm.; log ε: 4.37, 3.77, and 4.27; NMR ($CDCl_3$–tetramethylsilane): τ −4.10 (1H, s), 4.06 (1H, s), 5.12 (1H, t, J = 9 Hz.), 6.82 (2H, d, J = 9 Hz.), 6.84 (2H, t, J = 7 Hz.), 7.15 (2H, t, J = 7 Hz.), 7.30 (1H, s), 8.0–8.7 (4H, m), 8.53 (3H, s), 8.70 (3H, s), and 8.97 (6H, t).

Anal.—Calc. for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00. Found: C, 67.81; H, 7.22.

Epoxide of Mammein Diacetate—A solution of mammein diacetate (1.5 g., 3.3 mmoles) in chloroform (5 ml.) was added dropwise to an ice-cooled solution of monoperphthalic acid (910 mg., 4.95 mmoles) in ether (7 ml.). The reaction mixture was stirred for 5 hr. at 0° and then allowed to stand for 5 hr. at −15°. Phthalic acid was separated by filtration and washed with chloroform. The combined filtrate and chloroform washings were washed with saturated aqueous sodium bicarbonate solution (6 × 20 ml.), water (3 × 20 ml.), and saturated aqueous sodium chloride solution (2 × 20 ml.) and dried with anhydrous magnesium sulfate. Evaporation of the solvent gave a crystalline white solid, m.p. 93–95°; ν_{max}^{KBr}: 2950, 1754, 1698, 1372, 1208, 1192, 1110, and 1093 cm.$^{-1}$. Recrystallization of this material from aqueous ethanol gave white needles, 1.16 g. (75%), m.p. 95–97°; ν_{max}^{KBr}: 2959, 1779, 1730, 1715, 1385, 1372, 1200, 1176, 1166, 1112, 1096, and 1054 cm.$^{-1}$. The IR spectra of the crude product and the once recrystallized product differed substantially; however, their IR solution spectra and NMR spectra were identical, indicating that this epoxide exists in two crystalline modifications. $\nu_{max}^{CHCl_3}$: 2959, 1779, 1739, and 1701 (sh) cm.$^{-1}$; NMR ($CDCl_3$–tetramethylsilane): τ 3.72 (1H, s), 7.0–7.4 (8H, m), 7.55 (3H, s), 7.69 (3H, s), 8.0–8.6 (5H, m), 8.65 (3H, s), 8.72 (3H, s), and 8.8–9.15 (6H, m).

Anal.—Calc. for $C_{36}H_{32}O_8$: C, 66.08; H, 6.83. Found: C, 66.35; H, 6.74.

Cytotoxic Assay—The Sarcoma 180 tumor cells, maintained in T-type (T-60) flasks at 37°, were inoculated into T-15 flasks on Day 0. The cells, originally isolated by Foley and Drolet (21), were grown in Eagle's medium (22) in stationary culture. All assays were conducted in this medium with the following additional agents: L-glutamine (10 ml. of a solution 0.2 *M*), horse serum (100 ml./l.), and kanamycin (100 mg./l.).

Two hundred thousand cells were inoculated per 2 ml. of medium; following 24-hr. attachment of the cells to the glass of the flask, the test compounds were administered. The compounds either dissolved completely or remained partially suspended in the medium. Serial dilutions of the compounds were also given on the 4th day after initial transplant. Each dilution was run in triplicate, as was the control saline group. Culture media were changed on the day of compound administration, and the cytotoxic effect was estimated on the basis of cell growth as determined by total protein analysis conducted on Day 5 (23).

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Constituents of *Mammea americana* L. IX: Oxidation of Mammein and Mammeisin

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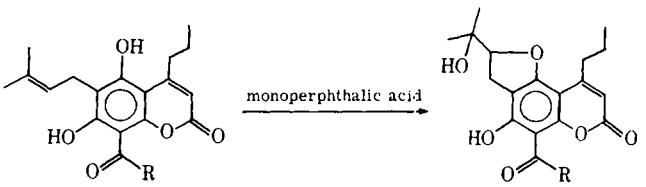
Abstract □ Cyclomammein, a putative mamey seed oil constituent, which has been obtained from mammein by treatment with peracids, was also produced upon air oxidation of the latter. In addition, two other air oxidation products of mammein were characterized. Both of these were also isolated by chromatography of the crude mammein mixture and, in common with cyclomammein, may be artifacts. One of these, designated M-9, $C_{22}H_{28}O_6$, m.p. 217–219°, is a linearly fused chromano coumarin; the other, L-2, $C_{22}H_{26}O_7$, m.p. 181–182°, contains a novel cyclic peroxide grouping and can be converted to cyclomammein by catalytic hydrogenation. The structures of these compounds were assigned on the basis of NMR and mass spectral measurements. The oxidation of mammein and mammeisin with dichlorodicyanobenzoquinone was also carried out. This leads to the corresponding chromeno coumarins: in the latter case, the product proved, as expected, to be identical with another mamey oil constituent, mammeigin. Finally, treatment of mammeisin with peracid leads to the derived dihydrofurano coumarin.

Keyphrases □ *Mammea americana* L.—oxidation of mammein and mammeisin □ Mammein and mammeisin—oxidation, isolation, and characterization of cyclomammein, chromeno and dihydrofurano coumarins □ Cyclomammein formation—oxidation of mammein □ Coumarins, chromeno and dihydrofurano formation—oxidation of mammein and mammeisin □ Oxidation of mammein and mammeisin—isolation and characterization of products □ NMR spectroscopy—identification, mammein and mammeisin oxidation products □ Mass spectroscopy—identification, mammein and mammeisin oxidation products

The preceding article (1) reported on the occurrence of a new series of *Mammea* coumarins (IV, V, and VI), which are related to mammein (I), neomammein (II), and normammein (III) in that the isopentenyl substituent at C-6 in the latter three molecules has undergone oxidative cyclization with the C-5 hydroxyl group to produce the hydroxyisopropyl dihydrofurano moiety



I: R = isobutyl
II: R = *sec*-butyl
III: R = *n*-propyl

IV: R = isobutyl
V: R = *sec*-butyl
VI: R = *n*-propyl

Scheme I

which is present in the former three, named cyclomammein (IV), cycloneomammein (V), and cyclonormammein. (VI). The peracid oxidation of I, II, and III provided a convenient partial synthesis of IV, V, and VI, as illustrated in Scheme I. This article discusses the details of further studies on the oxidation of mammein (I) and mammeisin (XX).

DISCUSSION

Peracid Oxidation of Mammein: Mammein Epoxide and Compound M-9—While the reaction illustrated in Scheme I proceeded cleanly in good yield, treatment of I[1] with *m*-chloroperbenzoic acid for 5 hr. at 0° provided IV in only 29% yield. However, in addition to IV, there was isolated a second substance, isomeric with IV, in 14% yield. This new product proved to be identical with a compound called M-9, isolated several years ago[2] from mammein

[1] In the reactions described in this paper, the mammein (I) used as starting material was accompanied by small amounts of its difficultly separable congeners, II and III. Accordingly, the products are similarly "contaminated." The properties of the mammein mixture (I, with II and III) are discussed in *Reference 1*.

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