2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-propylthiopyrrolidine was isolated as needles from chloroform–hexane: m.p. 123–124° (35% yield); R_f 0.95; infrared λ_{max}^{KBr} 3.05, 3.30 (broad), 3.45 (broad), 3.75 (broad), 6.20, 6.30, 6.62 μ.

Anal. Calcd. for $C_{15}H_{23}NO_2S$: C, 63.05; H, 8.25; N, 4.98; S, 11.77. Found: C, 63.49; H, 8.25; N, 5.22; S, 11.37.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-*n*-butylthiopyrrolidine was isolated as white needles from chloroform–hexane: m.p. 100–101° (78% yield); R_f 0.95; infrared λ_{max}^{KBr}3. 05, 3.42 (broad), 3.75 (broad), 6.18, 6.30, 6.60 μ.

Anal. Calcd. for $C_{16}H_{25}NO_2S$: C, 65.06; H, 8.53; N, 4.74; S, 10.83. Found: C, 65.33; H, 8.51; N, 4.60; S, 10.64.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-phenylthiopyrrolidine was isolated as needles from chloroform–hexane: m.p. 106–108° (56% yield); R_f0. 98; infrared λ_{max}^{KBr} 3.05, 3.12, 3.3 (broad), 3.45 (broad), 3.8 (broad), 6.20, 6.30, 6.62 μ.

Anal. Calcd. for $C_{18}H_{20}NO_2S$: C, 68.55; H, 6.71; N, 4.44; S, 10.15. Found: C, 68.85; H, 6.58; N, 4.13; S, 10.10.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-benzylthiopyrrolidine was isolated as white needles from chloroform–hexane: m.p. 122° (45% yield); R_f 0.98; infrared λ_{max}^{KBr} 2.95 (broad), 3.05, 3.30 (broad), 3.45 (broad), 3.75 (broad), 6.20, 6.30, 6.61, 6.70 μ.

Anal. Calcd. for $C_{19}H_{23}NO_2S$: C, 69.28; H, 7.04; N, 4.25; S, 9.71. Found: C, 69.38; H, 6.97; N, 4.24; S, 9.92.

Hydrolysis of 2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-acetoxypyrrolidine (XXIIa).—The acetoxy XXIIa (1.0 g.) was suspended in 25 ml. of 2 *N* sodium hydroxide solution. The mixture was warmed to dissolve the acetoxy compound, then refluxed for 2 hr. White needles, m.p. 172–173°, separated from the cooled reaction mixture; the yield was 500 mg. (60%). The needles were recrystallized twice from acetone: m.p. 172.5–173.5°, mixture melting point with an authentic sample of deacetylanisomycin (II) gave no depression; $[\alpha]^{25}D$ −20°, identical with II; infrared λ_{max}^{KBr} 3.0, 3.1, 6.22 μ, identical with the spectrum of deacetylanisomycin (II).

Hydrolysis of 2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-methoxypyrrolidine.—The methoxy compound XXIV (R = OCH_3), 500 mg., was heated for 5 hr. with boiling 48% hydrobromic acid. Evaporation of the reaction mixture gave a brown solid which was dissolved in hot 2-propanol, treated with activated charcoal, filtered, and allowed to crystallize. 2-*p*-Hydroxyphenylmethyl-3,4-*trans*-dihydroxypyrrolidine hydrobromide was obtained (390 mg., 63%), m.p. 201–204°, identical with material obtained by a similar hydrolysis of anisomycin (I): infrared λ_{max}^{KBr} 2.96, 3.04, 6.23, 6.29, 6.34 μ.

Constituents of *Mammea americana* L. IV. The Structure of Mammeigin[1,2]

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Mammeigin, $C_{25}H_{24}O_5$, a new 4-phenylcoumarin isolated from the seed oil of *Mammea americana* L., is shown to be 5-hydroxy-6-isovaleryl-8,8-dimethyl-4-phenyl-2H,8H-benzo[1,2-*b*:3,4-*b'*]dipyran-2-one. This structural assignment is based not only on spectroscopic evidence, but also on a chemical interrelation of mammeigin with mammeisin (4-phenyl-5,7-dihydroxy-6-isovaleryl-8-isopentenylcoumarin). The isolation of mammeol, a diterpene alcohol, is also recorded.

Chemical examination of various parts of the mamey tree (*Mammea americana* L., family *Guttiferae*) received its impetus over a century ago in the report of de Grosourdy[4] on its insecticidal activity. A number of studies of this property of mamey seed extracts[5–8] culminated in the isolation[9] of a crystalline active principle, mammein, for which structure 1 was advanced[10] on the basis of degradative and spectroscopic[10,11] as well as synthetic evidence.[12] Subsequently, a second toxic[13] coumarin isolated from the fruit peelings was shown[14] to possess structure **2**, 4-phenyl-5,7-dihydroxy-6-isovaleryl-8-isopentenylcoumarin, later named mammeisin.[15] Recent papers from this laboratory have dealt with the constitution of mamey wax,[2b] and with the isolation of 2-hydroxyxanthone[2a]; the present article details the isolation and proof of structure of still another mamey oil constituent, mammeigin (**3**) (see Figure 1).

When mamey oil, from which the wax had been removed by precipitation with acetone,[2b] was chromatographed on aluminum oxide, a yellow semisolid material was eluted with a benzene–Skellysolve B (9:1) mixture. After an extensive series of recrystallizations from ether–hexane mixtures, a small amount of mammeigin, yellow needles, m.p. 144–146°, was obtained which gave microanalytical data for $C_{25}H_{24-26}O_5$. The phenolic hydroxyl group in mammeigin (**3**) was evident from the infrared absorption band at 3400 cm.$^{-1}$ (potassium bromide), the characteristic shift of the ultraviolet maxima in the presence of added alkali, and from the positive reaction (green color) with ferric chloride. The general similarity of the infrared and ultraviolet spectra of mammeigin to those of mammeisin,[14] in particular, the infrared bands at 773 and 708 cm.$^{-1}$ indicative of a monosubstituted phenyl group, allowed the supposition that we were dealing with a new phenolic 4-phenylcoumarin. This supposition received strong support from phytochemical considerations, since four of the six previously known

(1) This research was generously supported by Research Grants GM-11412 and RG-8004 from the Division of General Medical Sciences, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

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(3) This article is based on a portion of a thesis presented by W. H. M. to the Department of Chemistry, The Ohio State University, inpartial fulfillment of the requirements for the Ph.D. degree, June 1964. Preliminary communication of this work has been made.[2c]

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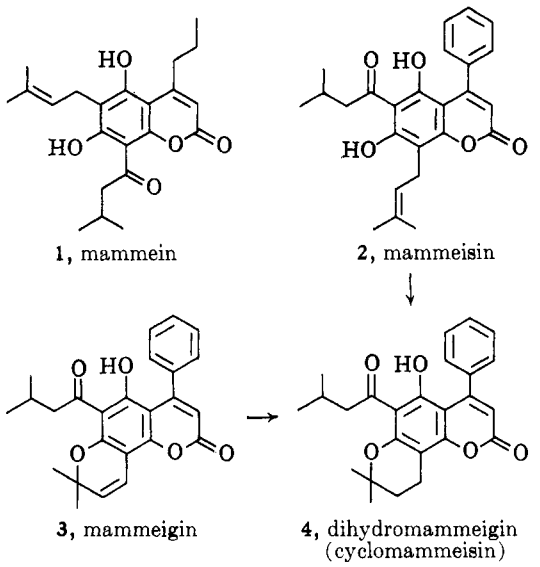


1, mammein 2, mammeisin

3, mammeigin 4, dihydromammeigin (cyclomammeisin)

Figure 1.

naturally occurring 4-phenylcoumarins[14,16-18] have been isolated from species of the same plant family, *Guttiferae*. Furthermore, these four[14,17,18] are oxygenated at positions 5 and 7 and three[14,17] of these four have isoprenoid units attached to positions 6 and 8.

Our initial hypothesis that mammeigin was simply the isomer of mammeisin (**2**), $C_{25}H_{26}O_5$, in which the positions of the isovaleryl and isopentenyl groups are reversed,[19] was dispelled immediately upon inspection of the n.m.r. spectra of these substances. In Figure 2, the spectrum of mammeisin is shown along with the proton assignments which clearly identify the various structural features present. In Figure 3 is similarly displayed the spectrum of mammeigin. The signals arising from the phenyl group (τ 2.94), the C-3 proton (τ 4.34), the acidic proton (τ −4.17, strongly hydrogen bonded), and the isovaleryl group (doublets at τ 7.14 and 9.06, multiplet about τ 8) have their counterparts in mammeisin (Figure 2); however, the absorptions expected for an isopentenyl group are lacking, and in addition, integration of the spectrum gives results in better accord with the H_{24} rather than the H_{26} formulation. The remaining signals at τ 3.33, 4.59, and 8.48 were recognized as those produced by a 2,2-dimethylchromene moiety. A number of examples which support this latter assignment appear in the literature and have been recently summarized by Stout and Stevens.[20]

This interpretation of the n.m.r. spectrum coupled with the other spectroscopic and microanalytical data, and including the presumptive evidence for a phloroglucinol oxygenation pattern, limit the structural possibilities for mammeigin to **3** and the isomers **5** and **6**. Catalytic reduction of **3** provided a dihydro derivative **4**, whose n.m.r. spectrum showed the replacement of the AB quartet in **3** by a pair of triplets (J = 6.8 c.p.s.), one centered at τ 7.13 and the other at 8.13 stemming from the pair of methylene groups of the chroman ring. The structure of **4** (and therefore of **3**) was secured when an identical substance was obtained from mammeisin (**2**) by an acid-catalyzed cyclization reaction (see Figure 1).[21] Further studies on the constituents of mamey seed extracts are in progress.

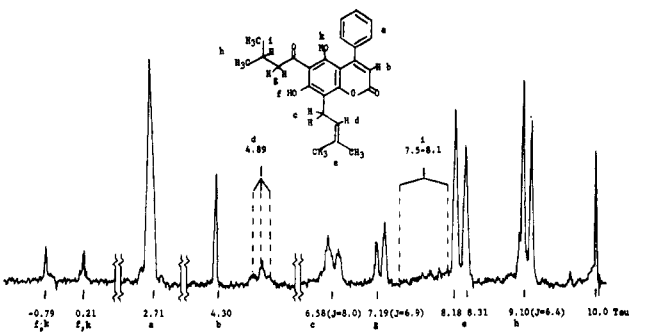


Figure 2.

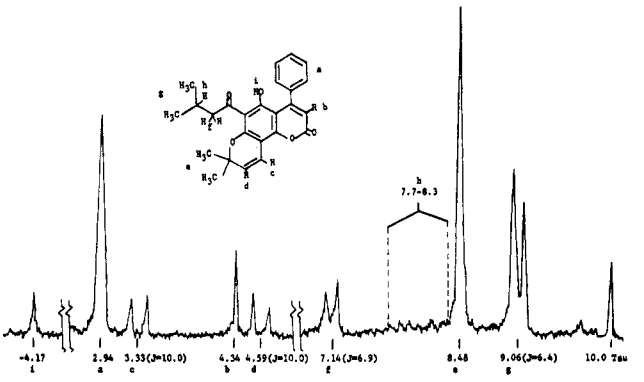
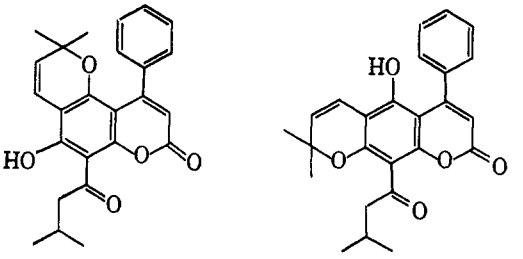


Figure 3.



5 6

Experimental

The infrared spectra were measured on a Perkin-Elmer Model 237 spectrophotometer while the ultraviolet spectra were recorded with a Cary Model 14 or a Perkin-Elmer Model 202 instrument. The n.m.r. spectra were determined with a Varian

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(19) Compare the structures of mammein and isomammein.[10]

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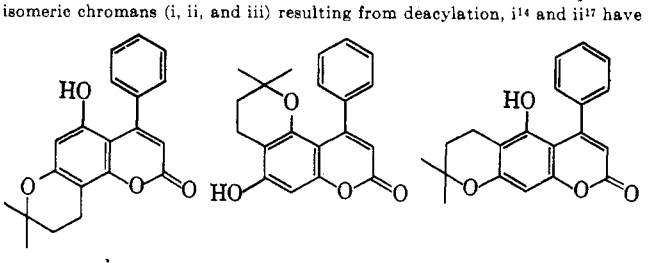
(21) It seems of interest to point out that, had this cyclization reaction either failed or had it succeeded but given a substance different from dihydromammeigin, the choice among the three isomeric possibilities, **3**, **5**, and **6**, could have been made following the application of the acid-catalyzed deacylation[10,12,14] reaction to dihydromammeigin. Of the three possible isomeric chromans (i, ii, and iii) resulting from deacylation, i[14] and ii[17] have



i ii iii

been described in the literature and structure iii had been provisionally assigned to a compound produced from i by isomerization (R. A. Finnegan and C. Djerassi, unpublished results). Naturally, the failure to link **3** with any of these isomers would have invalidated the assumption of a phloroglucinol-based nucleus in mammeigin.

Associates A-60 spectrometer, using deuteriochloroform as solvent and tetramethylsilane as internal reference. The melting points were taken on a Fisher-Johns block and are uncorrected. Microanalyses were performed in the laboratory of Dr. A. Bernhardt, Mülheim, Germany. All solvents were distilled before they were used.

Isolation.—Extraction of 75 lb. of dry seeds of *Mammea americana* L. with Skellysolve B provided 1.6 kg. of mamey oil after the removal of all acetone-insoluble material. Twenty grams of this extract was chromatographed on 1375 g. of aluminum oxide (Merck, acid washed). The column was eluted with Skellysolve B using an increasing ratio of benzene. Fractions of 500 ml. were taken.

Mammeol.—The first crystalline compound, mammeol, was obtained from a yellow wax (fractions 135–151), eluted with Skellysolve B–benzene (1:1). Several crystallizations of this material from hexane afforded 15 mg. (3.55×10^{-3}% yield, based on dry seeds) of white crystals: m.p. 148–150°; ν_{max}^{KBr} 3450, 2970, 1455, 1378, 1066, 968, and 979 cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{34}O$: C, 82.69; H, 11.80; O, 5.51; mol. wt., 290. Found: C, 82.29, 82.87; H, 11.69, 11.89; O, 5.56; mol. wt., 320.

Mammeigin (3).—From fractions 210–219, eluted with Skelly solve B–benzene (1:9), 237 mg. of a partly crystallized yellow oil was obtained. Separation of the crystalline material from the oil on an aluminum oxide column (Woelm, neutral, activity I) proved futile. Up to 30 crystallizations from ether–hexane mixtures afforded 58 mg. (1.37×10^{-2}% yield, based on dry seeds) of yellow needles: m.p. 144–146°; ν_{max}^{KBr} 3440 (broad), 1746, 1644, 1613, 1126, 773, 752, and 708 cm.$^{-1}$; $\lambda_{max}^{95\%\ ethanol}$ 234, 286, and 365 mμ (log ε 4.45, 4.52, and 4.11); $\lambda_{max}^{95\%\ ethanol-NaOH}$ 218, 251, 312, and 438 mμ (log ε 4.33, 4.38, 4.41, and 3.84). The compound gave a green color with ferric chloride.

Anal. Calcd. for $C_{25}H_{24}O_5$: C, 74.24; H, 5.98; O, 19.78; mol. wt., 404. Found: C, 74.34, 74.04; H, 6.15, 6.34; O, 19.81; mol. wt., 401.

The n.m.r. spectrum is reproduced in Figure 3. The relative integral was measured as follows: τ −4.17 (1), 2.94 (5.4), 3.33 (1.1), 4.34 (1), 4.59 (0.8), 7.14 (2.1), 7.72–8.33 (0.8), 8.48 (6.3), and 9.06 (6.2).

Dihydromammeigin (4) from Mammeigin (3).—Mammeigin (50 mg.) was stirred in 3 ml. of ethanol with 5% palladium on carbon (10 mg.) in a hydrogen atmosphere at 25°. The compound was not completely soluble in the amount of solvent which was limited by the size of the microhydrogenator. After 2 hr. 0.4 molar equiv. of hydrogen was taken up and further consumption occurred at a very slow pace. Ethanol was removed by a nitrogen jet and replaced by 3 ml. of tetrahydrofuran (distilled from $LiAlH_4$). Additional catalyst (5 mg.) was introduced and the hydrogen uptake ceased after 20 min. The total uptake was 0.97 molar equiv. Filtration of the reaction mixture and evaporation of the solvent afforded a yellow semisolid. Crystallization from a tetrahydrofuran–hexane mixture yielded 42 mg. of tan crystals, m.p. 152–160°. Three additional recrystallizations raised the melting point to 164–165° (25 mg.). From mother liquors, 11 mg., m.p. 154–161°, was obtained. A mixture melting point with a sample of dihydromammeigin, m.p. 165–166°, obtained on cyclization of mammeisin showed no depression, m.p. 164–166°. The infrared spectra of the two samples were superimposable, and their n.m.r. spectra showed identical shifts and coupling constants.

Dihydromammeigin (4) from Mammeisin (2).—A sample of 250 mg. of mammeisin was dissolved in 5 ml. of glacial acetic acid and 3 drops of concentrated sulfuric acid was added. After standing for 18 hr. at room temperature, the reaction mixture was poured onto ice–water. A tan amorphous material precipitated. The filtered solid was dissolved in ether, washed several times with water, and dried over magnesium sulfate. Removal of the ether afforded 233 mg. of tan crystals, m.p. 146–158°. Three further recrystallizations from tetrahydrofuran–ether–hexane mixtures yielded tan needles: m.p. 165–166°; ν_{max}^{KBr} 3436 (broad), 1745, 1730 (sh), 1613, 1587 (sh), 1124, 770, and 714 cm.$^{-1}$; $\lambda_{max}^{95\%\ ethanol}$ 287 and 341 mμ (log ε 4.54 and 4.05); $\lambda_{max}^{95\%\ ethanol-NaOH}$ 293, 321, and 420 mμ (log ε 4.07, 4.12, and 3.89).

Anal. Calcd. for $C_{25}H_{26}O_5$: C, 73.87; H, 6.45; O, 19.68. Found: C, 73.78; H, 6.60; O, 19.56.

Acknowledgment.—The technical assistance of Mrs. U. Mueller, Mr. G. Hogsten, and Mr. B. A. Modi is gratefully acknowledged.

Analogs of Firefly Luciferin

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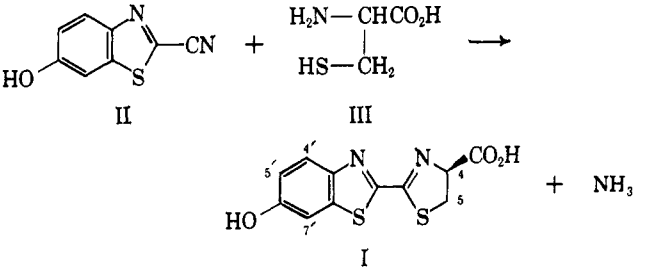
The synthesis of firefly luciferin (and the C^{14}- and S^{35}-labeled forms) is outlined. In addition, the syntheses of O-methylluciferin, 5,5-dimethylluciferin, deshydroxyluciferin, decarboxyluciferin, and a pyridine analog are reported.

Recently we showed by degradation and synthesis that luciferin, the oxidizable substrate responsible for light emission in the firefly, is D-(−)2-(6′-hydroxy-2′-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic acid (I).[1] We have since synthesized a number of analogs in an effort to determine the structural requirements for firefly luciferin in bioluminescence; these syntheses are the subject of this paper.

Firefly Luciferin (I).—The final and key step in our earlier synthesis of luciferin[1] was the condensation of 2-cyano-6-hydroxybenzothiazole (II) with cysteine (III); aqueous methanol was used as the solvent, and the condensation was effected at room temperature.[2] Compound II was prepared, in turn, by



the demethylation of 2-cyano-6-methoxybenzothiazole (IV). This compound has now been prepared by three different routes. Method 1, requiring six steps, has been largely replaced by method 3 in which a commercially available intermediate containing the benzothiazole nucleus is used. Method 3 also lends itself to the synthesis of C^{14}-containing luciferin (see Experimental) since potassium cyanide-C^{14} is readily available. Method 2, introduced recently by Japanese

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(2) A similar condensation has also been reported by H. Baganz and L. Domaschke, *Ber.*, **95**, 1842 (1962).