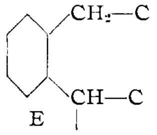
fractions split into a lower and higher boiling fraction again arbitrarily. Infrared spectral examination of the six distillation fractions revealed strong bands for three different types of benzene ring substitution. Bands for hydroxyl, carbonyl and other oxygen-containing groups were totally absent. No one distillation fraction appeared homogeneous on the basis of its spectrum. Comparisons of the spectra with many reference spectra[20] allowed allocation of minimum structural features to each of the three substituted benzenes, E-G.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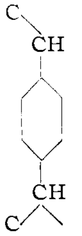
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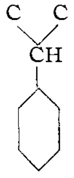
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Separation of the selenium dehydrogenation products was effected by vapor phase chromatography,[21] which revealed the presence of no fewer than six, and probably eight, benzenoid compounds bearing C_4-, C_5-, C_7-, C_8- and C_9-alkyl groups (in the one or two side chains). In view of the observed complexity no further work was performed on these reaction products.

Action of Acid on Dihydroalcohol Ia.—Dihydroalcohol Ia (1.06 g.) was dissolved in dioxane (20 ml.), the stirred solution treated with sulfuric acid (20 ml. of 10%) and boiled under reflux for 3 hours in a nitrogen atmosphere. The cold reaction solution, after neutralization with bicarbonate, was diluted to 100 ml. and the majority of the dioxane removed by distillation, prior to extraction with three 50-ml. portions of ether. Evaporation of the dried ether extracts yielded a brown oil (0.90 g.), separated by chromatography on alumina (25 g.) into two components. Elution with 100:1 ether-methanol removed a pale yellow gum (0.16 g.), $[\alpha]_D$ 39.5°, showing hydroxyl but no carbonyl in the infrared. After distillation the gum was colorless, b.p. 119-126° (bath) (5×10^{-4} mm.).

Anal. Calcd. for $C_{15}H_{28}O_4$: C. 66.14; H, 10.36; O, 23.50. Found: C, 66.20; H, 10.03; O, 23.83; active hydrogen, 0.56.

A pale yellow gum was eluted with 30:1 and 20:1 ether-methanol which, on distillation, was separated into a lower and a higher boiling fraction. The fraction b.p. 128-136° (bath) (5×10^{-4} mm.) was a colorless gum.

Anal. Calcd. for $C_{16}H_{30}O_5$: C, 63.54; H, 10.00; O, 26.46; active hydrogen, 3. Found: C. 64.03; H, 9.97; O, 26.26; active hydrogen, 2.58.

The second fraction, b.p. 149-162° (bath) ($5 \times 10^{-}$ mm.), was also a colorless gum.

Anal. Calcd. for $C_{16}H_{30}O_5$ (see above): Found: C, 63.30; H, 10.01; O, 26.57.

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[20] American Petrolum Institute Infrared Tables.

[21] We are indebted to Prof. A. C. Cope of Massachusetts Institute of Technology for the separation. A silicone oil column was used at 265° with a preheater at 300°

[JOINT CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY AND THE UNITED STATES DEPARTMENT OF AGRICULTURE FEDERAL EXPERIMENT STATION IN PUERTO RICO]

Naturally Occurring Oxygen Heterocyclics. II.[1] Characterization of an Insecticidal Principle from *Mammea americana* L.[2]

BY CARL DJERASSI, E. J. EISENBRAUN, B. GILBERT, A. J. LEMIN, S. P. MARFEY AND M. P. MORRIS

RECEIVED FEBRUARY 17, 1958

The crystalline insecticidal principle from the seeds of *Mammea americana* L. has been named mammein and found to possess the empirical formula $C_{22}H_{28}O_5$. Chemical and spectroscopic data are presented to support the characterization of the oxygen functions in terms of a lactone ring, a conjugated carbonyl moiety and two phenolic (or enolic) hydroxyl groups. The reactive double bond of mammein has been shown to be present in the system $(CH_3)_2C{=}CH{-}\overset{|}{\underset{|}{C}}{-}$.

The mamey tree, *Mammea americana* L., is indigenous to the West Indies and it has been known for some time that various parts of the plant possess insecticidal properties.[3] The seeds[4] of the fruit are particularly effective in this respect and while the latter is edible, reports of poisoning in humans are known. Recently,[5] one pure crystalline toxic constituent has been isolated from the seeds and assigned the empirical formula $C_{18}H_{22}O_4$. Earlier work[6] with crude seed extracts suggested a structural similarity to the pyrethrins, but the apparent empirical formula (for correction see below) and especially the reported[5] ultraviolet absorption maxima of the crystalline material clearly showed that this could not be the case.

In view of the fact that the important, naturally occurring insecticides[7] can be reduced to three distinct chemical types—rotenoids, pyrethrins and unsaturated isobutylamides—it was of obvious interest to determine whether the insecticidal activity of mamey was due to a different chemical type. If true, then this might offer a lead to the synthesis of new and possibly more effective insecticides. Our work has concentrated on the toxic crystalline constituent of the seeds isolated by Morris and Pagan[5] and we have coined the name "mammein" for it. The present paper is concerned with an improved isolation of mammein, the preparation of various derivatives and the tentative characterization of the functional groups.

The ground seed was extracted with petroleum ether, the resulting oil was treated with acetone to remove some insoluble material[5] and then was chromatographed on acetic acid-deactivated alumina.[8] Elution with petroleum ether and petroleum

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[1] The paper by W. B. Mors, O. R. Gottlieb and C. Djerassi, THIS JOURNAL, **79**, 4507 (1957), is considered Part I of this series.

[2] The work at Wayne State University was supported by a research grant from the National Science Foundation.

[3] See H. K. Plank, *J. Econ. Entom.*, **37**, 737 (1944).

[4] H. K. Plank, *Trop. Agric.*, **27**, 38 (1950).

[5] M. P. Morris and C. Pagan, THIS JOURNAL, **75**, 1489 (1953).

[6] M. A. Jones and H. K. Plank, *ibid.*, **67**, 2266 (1945).

[7] For review see L. Feinstein and M. Jacobson in L. Zechmeister's "Progress in the Chemistry of Organic Natural Products," Springer, Vienna, 1953, Vol. X, pp. 423-476.

[8] The lability of mammein toward alkali precluded the use of alkaline alumina as adsorbent.

ether–benzene mixtures followed by recrystallization afforded approximately 0.26% of mammein (m.p. 128.5–129.5°) based on dry seed. Identity with compound A (m.p. 130–131°) of Morris and Pagan[5] was demonstrated by direct comparison. The earlier reported[5] analytical figures for compound A ($C_{18}H_{22}O_4$) also correspond to $C_{22}H_{28}O_5$ and repeated analyses including molecular weight determinations have now shown that the latter formula is the correct one for mammein. Functional group analysis indicated the presence of three C-methyl groups and the absence of methoxyl functions. Mammein is optically inactive, gives a deep purple-brown color with ferric chloride and can be titrated with alkali in 66% dimethylformamide solution whereupon a pK_a' of 5.9 was observed. This value may correspond to a carboxylic acid or enolic function and for comparison the pK_a' of 4-hydroxycoumarin was determined at the same time and found to be 5.9. The ultraviolet absorption spectrum of mammein in acid solution, with a single maximum at 295 mμ, is drastically altered in alkaline solution (λ_{max} 232, 256 and 332 mμ), while its infrared spectrum (chloroform solution) shows hydroxyl absorption (3.03 μ) as well as two pronounced bands in the carbonyl region at 5.78 and 6.20 μ.

The presence of phenolic and/or enolic groups in mammein (I) was indicated by the positive ferric chloride reaction and this was confirmed by the ready formation of a diacetate (II), which no longer gave a color with ferric chloride and did not exhibit infrared hydroxyl absorption but rather a new strong band at 5.62 μ corresponding to phenolic (or enolic) acetates.[9] Similarly, methylation with dimethyl sulfate in acetone solution in the presence of potassium carbonate afforded mammein dimethyl ether (III) and as indicated in the Experimental section, its ultraviolet absorption spectrum is quite similar to that of mammein in acid solution. A difference in the reactivity of the two hydroxyl groups—probably due to hydrogen bonding—could be detected when the methylation of mammein (I) was undertaken with diazomethane. Although a small amount of the dimethyl ether III (no titratable group between pH 4–11) was isolated, the principal product proved to be mammein monomethyl ether (IV) (pK_a' 9.2) which gave a purple-brown color with ferric chloride solution but did not exhibit a hydroxyl band in the infrared, suggesting that the hydroxyl group was involved in hydrogen bonding.

The number of unsaturated linkages in mammein (I) could not be determined definitely. Quantitative microhydrogenation of mammein (I) with platinum oxide catalyst in glacial acetic acid resulted in the uptake of four molar equivalents of hydrogen in 16 hours, but hydrogen continued to be consumed at a very slow rate. On the other hand, when the hydrogenation was conducted in methanolic solution, hydrogen consumption stopped after the rapid up-take of one mole and dihydromammein (V) could be obtained in good yield. The double bond thus reduced was not involved in the chromophore of mammein (I) since the ultraviolet absorption spectrum (Fig. 1) of dihydromammein (V) was similar to that of mammein. Just as with mammein, dihydromammein (V) was characterized further by formation of a diacetate (VI)[10] and a dimethyl ether (VII) which no longer gave a color with ferric chloride solution. The double bond was not involved to any marked extent with the acidic function of mammein (I) since the pK_a' of dihydromammein (V) differed only slightly (6.1 *vs.* 5.9) from that of the parent compound. The reactivity of this double bond was demonstrated further by treatment of mammein (I) in ether solution with hydrogen chloride gas, which caused addition of hydrogen chloride to the double bond.

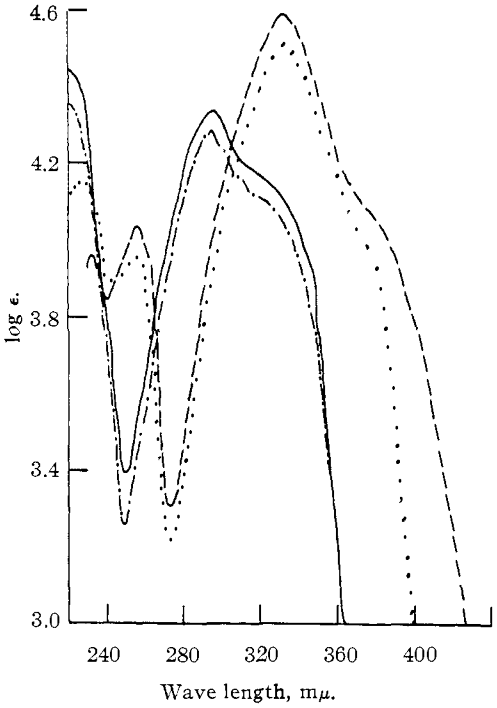

Fig. 1.—Ultraviolet and visible absorption spectra in ethanolic hydrochloric acid (pH 2): ———, mammein (I); —·—·—·, dihydromammein (V); in ethanolic potassium hydroxide (pH 13): _ _ _ _, mammein; ......, dihydromammein.

The nature of this reactive double bond was clarified further by ozonization experiments. Passage of a limited amount of ozone through an acetic acid solution of mammein (I) afforded acetone in 51% yield, while dihydromammein did not yield any volatile fragment upon similar treatment and a substantial amount of unreacted dihydromammein could be recovered. In the case of the ozonization of mammein (I), the non-volatile fragment XIV proved to be crystalline and apparently represented an aldehyde ($\lambda_{max}^{CS_2}$ 3.59 μ typical of aldehydes, positive Tollens reaction), the empirical formula ($C_{19}H_{22}O_6$) of which corresponded to simple oxidative fission of an isopropylidene function. The above experiments are, therefore, only com-

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[9] *Cf.* R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

[10] The ultraviolet absorption spectra of dihydromammein diacetate (VI) and dimethyl ether (VII) were also virtually identical with those of the corresponding mammein derivatives (II, III).

patible with the partial structure $(CH_3)_2C{=}CH{-}\overset{|}{\underset{|}{C}}{-}$ which can be found in many naturally occurring plant products.

The reaction of mammein with alkali is complex and depending upon the reaction conditions can lead to rupture of the molecule. These experiments will be discussed in detail in a future paper, but the results of mild alkaline treatment are pertinent to the characterization of mammein and will be covered below.

Mammein can be dissolved in 5% potassium hydroxide solution and nearly 50% of it can be recovered unchanged after 15 minutes upon acidification. Upon longer standing at room temperature, acidification yields a yellow solid, isomeric with mammein and which has been named isomammein (VIII). Its ultraviolet absorption spectrum (Fig. 2) differs markedly from that of mammein thus demonstrating that the main chromophore must have been affected in some manner. The infrared spectra (chloroform solution) of mammein (I) and isomammein (VIII) differ not only in the fingerprint region, but the 6.20 μ band of mammein occurs now as a doublet (6.11 and 6.23 μ) in isomammein. Furthermore, the pK'_a of the latter (5.2) differs considerably from that of mammein (5.9). Nevertheless, the main functional groups identified above in mammein are also present in isomammein. Thus, isomammein forms a diacetate X and a dimethyl ether IX and it can be hydrogenated to dihydroisomammein (XI), which is also obtainable by alkaline treatment of dihydromammein (V). The intact hydroxylic functions of mammein are required for this isomerization since dihydromammein dimethyl ether (VII) is not affected by the same alkaline conditions. The double bond of isomammein (VIII) occupies the same position as in mammein (I) since ozonization of the former affords acetone in 60% yield together with a crystalline, yellow aldehyde XV analogous to the colorless one XIV obtained in the ozonization of mammein (I).

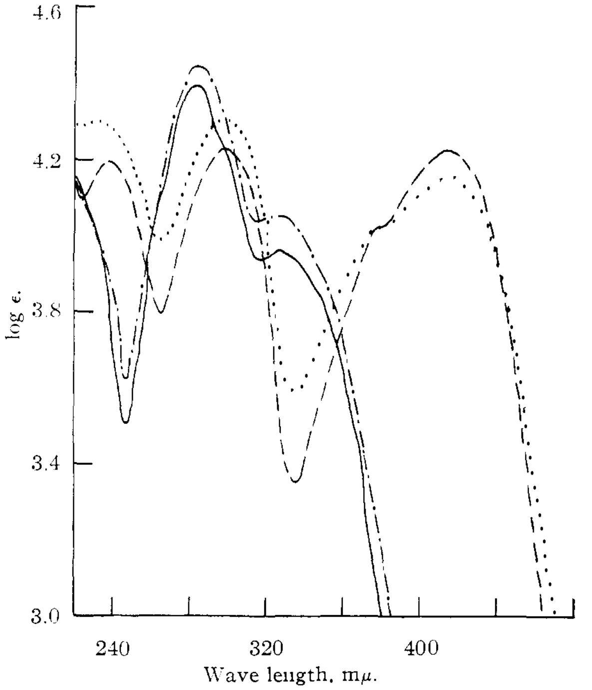

Fig. 2.—Ultraviolet and visible absorption spectra in ethanolic hydrochloric acid (*p*H 2): ———, isomammein (VIII); —·—·—·, dihydroisomammein (XI); in ethanolic potassium hydroxide (*p*H 13): --------, isomammein; ......, dihydroisomammein.

Of the five oxygen atoms present in mammein, two have been identified as phenolic (or enolic) hydroxyl groups by formation of appropriate derivatives. Since these derivatives (diacetate or dimethyl ether) do not exhibit any hydroxyl absorption in the infrared, the remaining three oxygen atoms must be represented by carbonyl or ether functions. Mammein and its derivatives do not contain a free carboxyl group, but their behavior toward alkali indicates the presence of an acidic moiety. That this is most likely a lactone could be demonstrated by the fact that mammein dimethyl ether (III) or dihydromammein dimethyl ether (VII) can be dissolved by warming in dilute potassium hydroxide solution and are extractable from such solution with ether only upon acidification. Of the two infrared bands of mammein in the carbonyl region, only the one at 5.78 μ can be due to the lactone and its position suggests an α,β-unsaturated 5-membered lactone[11] or possibly a coumarin system.[12] The second one at 6.20 μ is most likely due to a carbonyl function (in conjugation with an unsaturated or aromatic moiety), superimposed on an aromatic absorption band[12] thus accounting for all five oxygen atoms. Attempts to prepare a carbonyl derivative failed since no pure product could be isolated, but in no instance was the starting material recovered.

The precise nature and location of the functional groups as well as the nuclear skeleton will become apparent from degradative and synthetic reactions now in progress.

Experimental[13]

Isolation of Mammein (I).—The fruits of *Mammea americana* L. were collected in the outskirts of Mayaguez, Puerto Rico; the seeds were separated and promptly dried and ground. In several instances, such dried, ground material was stored for several months before being processed further.

A 100-kg. batch of ground seeds was extracted[14] exhaustively by percolation with petroleum ether (b.p. 30–60°) and the extract was concentrated to a brown, mobile oil weighing 5.82 kg. A sample (200 g.) of this oil was further concentrated on the steam-bath *in vacuo* which reduced the weight to 162 g. Acetone (800 cc.) was then added and filtration of the flocculent precipitate through Filter-Cel and removal of the acetone yielded 150 g. of dark, brown oil.

Chromatography of 100 g. of oil on 1.5 kg. of deactivated alumina[15] and elution with petroleum ether removed a large

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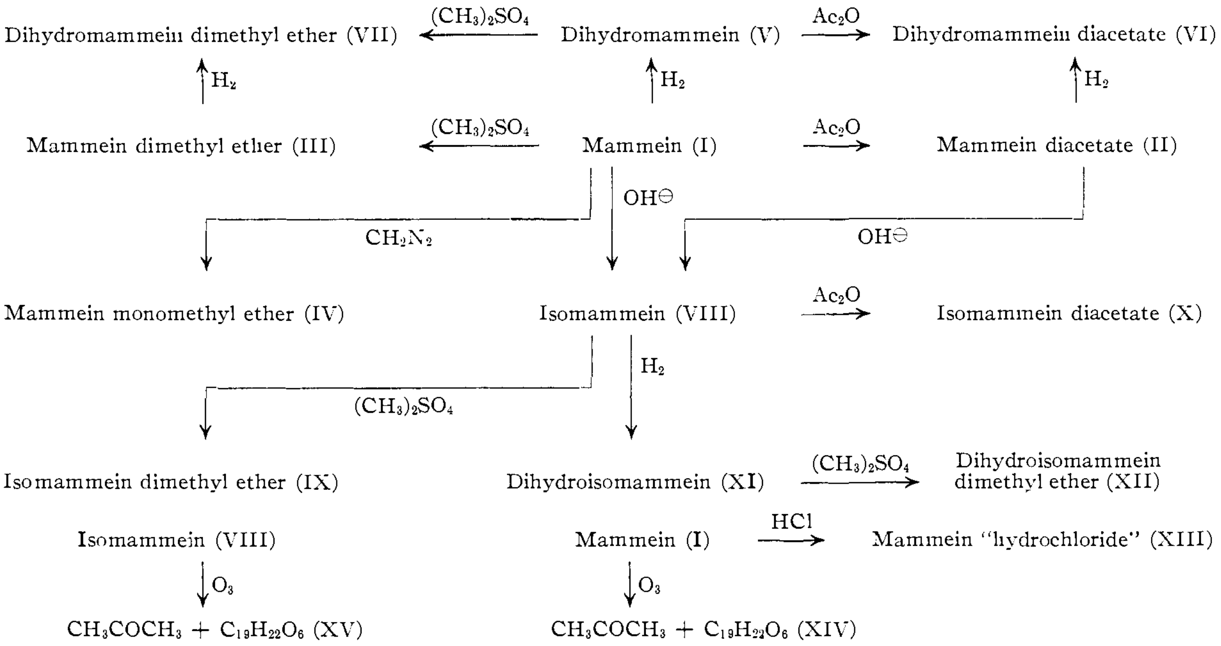
[11] See, for instance, J. F. Grove and H. A. Willis, *J. Chem. Soc.*, 877 (1951).

[12] Coumarin, 7- and 8-methoxycoumarin show infrared bands in chloroform solution at 5.80 and 6.21 μ.

[13] Melting points were determined on the Kofler block. We are indebted to Mrs. Dolores Phillips for the infrared and ultraviolet spectral measurements and to Dr. A. Bernhardt (Mülheim, Germany) for the microanalyses. All electrometric titrations were carried out in the Eli Lilly Research Laboratories (Indianapolis, Ind.) through the courtesy of Dr. H. Boaz.

[14] We are greatly indebted to Drs. John Controulis and Wayne Donaldson for carrying out this extraction in the pilot plant of Parke, Davis and Co., Detroit, Mich.

[15] The alumina (Alcoa, grade F-20) was pretreated by suspending in petroleum ether and shaking with 150 cc. of 10% aqueous acetic acid



quantity (41 g.) of yellow oil. Further development of the chromatogram with petroleum ether and petroleum ether–benzene (3:1) gave 5.5 g. of colorless mammein (after trituration with petroleum ether to remove adhering oil). Recrystallization from aqueous methanol and from chloroform–hexane led to 4.12 g. (0.26% over-all yield based on dry seed) of mammein, m.p. 124–128°, which was satisfactory for further work. Homogeneity was established by descending paper chromatography on formamide-impregnated Whatman No. 1 paper using cyclohexane saturated with formamide.[16] The single spot on the paper strip due to the presence of mammein was detected by irradiation with ultraviolet light on a zinc sulfide screen.

The analytical sample was obtained from hexane and the transparent prisms exhibited the following properties: m.p. 128.5–129.5°, dark purple-brown color with alcoholic ferric chloride solution, no perceptible rotation in chloroform solution; $\lambda_{max}^{CHCl_3}$ 3.03(m), 5.78(s), 6.20(s) and 6.37(m) μ; $\lambda_{max}^{CS_2}$ 3.02(m), 5.71(s), 5.82(sh), 6.25(s) μ. The ultraviolet absorption spectrum (Fig. 1) showed $\lambda_{max}^{EtOH-HCl}$ 295 mμ (log ϵ 4.34), $\lambda_{min}^{EtOH-HCl}$ 250 mμ (log ϵ 3.39), $\lambda_{max}^{EtOH-KOH}$ 232, 256 and 332 mμ (log ϵ 3.97, 4.04, 4.60), $\lambda_{min}^{EtOH-KOH}$ 240 and 274 mμ (log ϵ 3.84, 3.30).

Anal. Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58; 3 C–CH_3, 12.11; mol. wt., 372.4. Found: C, 70.84, 70.85; H, 7.49, 7.70; C–CH_3, 10.43; OCH_3, 0.0; mol. wt., 394 (Rast), 366 (electrometric titration in 66% DMF, initial *p*H 4.3, *pK*$_a'$ 5.9).

Mammein Diacetate (II).—A mixture of 0.12 g. of mammein, 1 cc. of acetic anhydride and 6 cc. of pyridine was left overnight at room temperature, diluted with water and extracted with ether. Evaporation of the washed ether extract to dryness and crystallization from petroleum ether furnished 0.1 g. of crystals (no color with ferric chloride), m.p. 101.5–102.5° raised to 105–107.5° after repeated recrystallization from the same solvent. The analytical sample exhibited $\lambda_{max}^{CHCl_3}$ 5.62(s), 5.74(s), 5.83 (sh), 6.15(m), 6.23(m). 6.33(m) μ and the ultraviolet absorption spectrum (λ_{max}^{EtOH} 281 and 320 mμ, log ϵ 4.10 and 3.82; λ_{min}^{EtOH} 256 and 308 mμ, log ϵ 3.88 and 3.78) showed some similarity to that of coumarin (λ_{max}^{EtOH} 274.5 and 312.5 mμ, log ϵ 4.20 and 3.91; λ_{min}^{EtOH} 242.5 and 300 mμ, log ϵ 3.55 and 3.86).

Anal. Calcd. for $C_{26}H_{32}O_7$: C, 68.40; H, 7.07. Found: C, 68.65; H, 7.08.

Mammein Dimethyl Ether (III).—Anhydrous potassium carbonate (5 g.) and dimethyl sulfate (4 cc.) were added to a solution of mammein (5 g.) in dry acetone (150 cc.). The yellow solution was stirred and heated under reflux for 1.5 hr. Potassium carbonate (2 g.) and dimethyl sulfate (1 cc.) were then added and refluxing continued for a total of 24 hr. The solution was filtered and the acetone evaporated giving a yellowish solid (5.7 g.). This was dissolved in ether (100 cc.) and the ethereal solution washed with 2 *N* sodium hydroxide solution (50 cc.), twice with water, dried ($MgSO_4$) and evaporated, giving a yellowish solid (5.2 g.), which after recrystallization from ethanol gave crude mammein dimethyl ether, m.p. 84–93° (4.0 g., 74%). Further recrystallization from ethanol and from hexane raised the melting point to 103–103.5°.

The compound gave no color with ferric chloride and showed no titratable groups in 66% DMF; $\lambda_{max}^{CHCl_3}$ 5.77(s), 6.19(m), 6.29(s) μ, $\lambda_{max}^{CS_2}$ 5.69 and 5.81 μ. The ultraviolet absorption spectrum showed λ_{max}^{EtOH} 295 mμ (log ϵ 4.13) and λ_{min}^{EtOH} 260 mμ (log ϵ 3.80) and was not altered by the addition of alkali.

Anal. Calcd. for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05; 2 OCH_3, 15.50; 3 C–CH_3, 11.26. Found: C, 72.28; H, 8.19; OCH_3, 15.28; C–CH_3, 10.68.

Mammein Monomethyl Ether (IV).—Diazomethane from *p*-toluenesulfonylmethylnitrosamide (11.5 g.) was distilled with ether (67 cc.) into a solution of mammein (5 g.) in dry acetone (100 cc.) at 0°. The solution became red then yellow and vigorous evolution of gas occurred. After 24 hr. at room temperature the solution was evaporated to give a yellow oil (5.4 g.). Chromatography on Merck acid-washed alumina (80 g.) gave an initial crystalline fraction (3.6 g.) eluted with hexane and 1:1 hexane–benzene. Crystallization from hexane gave mammein monomethyl ether as pale yellow needles (1.9 g., 37%) which after four more crystallizations had m.p. 80–82°. The compound gave a purple-brown color with alcoholic ferric chloride; $\lambda_{max}^{CHCl_3}$ 5.81(s), 6.18(s), 6.27(s), 6.37(m) μ, $\lambda_{max}^{CS_2}$ 5.71 μ. There was no distinct hydroxyl peak in the infrared absorption spectrum. The ultraviolet absorption spectrum showed λ_{max}^{EtOH} 286 and 318 mμ (log ϵ 4.29, 4.18) a broad shoulder at 340 mμ (log ϵ 4.14) and an inflection at 384 mμ (log ϵ 3.56), λ_{min}^{EtOH} 245.5 and 304 mμ (log ϵ 3.95, 4.14). $\lambda_{max}^{1\,N\ EtOH-KOH}$ 234 and 384 mμ (log ϵ 4.38. 4.40), $\lambda_{min}^{1\,N\ EtOH-KOH}$ 227.5 and 287 mμ (log ϵ 4.35, 3.31).

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.47; H, 7.82; O, 20.70; 1 OCH_3, 8.03; mol. wt., 386.5. Found: C, 71.28; H, 7.76; O, 20.60; OCH_3, 8.08; mol. wt., 407 (electrometric titration in 66% DMF, initial *p*H 7.0, *pK*$_a'$ 9.2).

In another experiment mammein dimethyl ether, m.p. 103–103.5° (22 mg.), was isolated.

Dihydromammein (V).—Mammein (200 mg.) in 15 cc. of methanol was hydrogenated in the presence of 30 mg. of platinum oxide catalyst at room temperature and atmospheric pressure until the hydrogen up-take ceased. After

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[16] R. B. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, **188**, 763 (1951).

working up in the usual way including passage over 10 g. of deactivated alumina and recrystallization from petroleum ether, there was obtained 150 mg. of dihydromammein (V), m.p. 132–133°, dark brown color with ferric chloride; $\lambda_{\max}^{\mathrm{CHCl_3}}$ 5.79(s), 6.20(s), 6.37(m) μ; $\lambda_{\max}^{\mathrm{CS_2}}$ 5.74(s), 5.85(s), 6.22(s) μ. The ultraviolet absorption spectrum is reproduced in Fig. 1 and showed $\lambda_{\max}^{\mathrm{EtOH-HCl}}$ 294 mμ (log ε 4.29), $\lambda_{\min}^{\mathrm{EtOH-HCl}}$ 249.5 mμ (log ε 3.25), $\lambda_{\max}^{\mathrm{EtOH-KOH}}$ 227, 255.5 and 332 mμ (log ε 4.16, 3.96, 4.52), $\lambda_{\min}^{\mathrm{EtOH-KOH}}$ 244 and 274 mμ (log ε 3.90, 3.22).

Anal. Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08; mol. wt., 374.5. Found: C, 70.75; H, 8.08; mol. wt., 400 (Rast), 375 (electrometric titration in 66% DMF, initial *p*H 4.6, pK_a' 6.1).

Dihydromammein Diacetate (VI). (a) By Hydrogenation of Mammein Diacetate (II).—Hydrogenation of 100 mg. of mammein diacetate (II) with platinum oxide in methanol solution followed by recrystallization from petroleum ether gave 90 mg. of dihydromammein diacetate (VI), m.p. 78.5–79.5°, raised to 86–87° after several additional recrystallizations; $\lambda_{\max}^{\mathrm{CHCl_3}}$ 5.61(s), 5.72(s), 5.82(sh), 6.15(m), 6.23(m) 6.32(m) μ. The ultraviolet absorption spectrum ($\lambda_{\max}^{\mathrm{EtOH}}$ 280 and 318 mμ, log ε 3.97 and 3.73; $\lambda_{\min}^{\mathrm{EtOH}}$ 255 and 306 mμ, log ε 3.78 and 3.70) was nearly identical with that of mammein diacetate (II).

Anal. Calcd. for $C_{26}H_{34}O_7$: C, 68.10; H, 7.47; acetyl, 18.87. Found: C, 68.09; H, 7.54; acetyl, 19.14.

(b) By Acetylation of Dihydromammein (V).—Acetylation of 45 mg. of dihydromammein with acetic anhydride–pyridine at room temperature produced 25 mg. of diacetate which was identified with material prepared according to (a) by mixture melting point determination and infrared comparison.

Dihydromammein Dimethyl Ether (VII). (a) By Hydrogenation of Mammein Dimethyl Ether (III).—Hydrogenation of 380 mg. of mammein dimethyl ether was accomplished in methanol solution with platinum oxide catalyst and led after recrystallization from petroleum ether to 290 mg. of dihydromammein dimethyl ether, m.p. 89–90°; $\lambda_{\max}^{\mathrm{CHCl_3}}$ 5.76(s), 6.16(m), 6.26(s) μ; $\lambda_{\max}^{\mathrm{EtOH}}$ 295 mμ (log ε 4.09) and $\lambda_{\min}^{\mathrm{EtOH}}$ 259.5 mμ (log ε 3.76).

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51; 2 OCH_3, 15.43. Found: C, 71.51; H, 8.65; OCH_3, 15.47.

(b) By Methylation of Dihydromammein (V).—A mixture of 420 mg. of dihydromammein, 620 mg. of anhydrous potassium carbonate, 0.21 cc. of dimethyl sulfate and 20 cc. of anhydrous acetone was heated under reflux with stirring for 4 hr. After dilution with water, extraction with ether, evaporation to dryness and recrystallization from petroleum ether, there was isolated 390 mg. of dihydromammein dimethyl ether, m.p. 86–87°, which proved to be identical with the specimen prepared by procedure (a). Attempted isomerization of dihydromammein dimethyl ether (VII) with 5% methanolic potassium hydroxide at room temperature yielded 90% of recovered starting material after acidification.

Mammein "Hydrochloride" (XIII).—Hydrogen chloride gas was passed for 1.5 hr. through an ethereal solution (40 cc.) of 130 mg. of mammein (I), the solvent was removed and the residue was recrystallized from petroleum ether to yield 100 mg. of water-insoluble crystals with a double m.p. 135–139° and 159–165°. The solid gave a dark brown color with ferric chloride solution and after further recrystallization from ether–petroleum ether exhibited a single m.p. 142–144°. The ultraviolet absorption spectrum ($\lambda_{\max}^{\mathrm{EtOH}}$ 220.5, 294 and 324.5 mμ, log ε 4.41, 4.26, 4.24; inflection at 258 mμ, log ε 3.71 and $\lambda_{\min}^{\mathrm{EtOH}}$ 249 and 309 mμ, log ε 3.60 and 4.21) closely resembled that of dihydromammein (V) in neutral solution showing that hydrogen chloride had added to the double bond.

Anal. Calcd. for $C_{22}H_{29}ClO_5$: C, 64.60; H, 7.15; Cl, 8.67. Found: C, 64.66; H, 7.45; Cl, 7.96.

Isomammein (VIII).—A solution of 1.0 g. of mammein in 35 cc. of methanol containing 1.9 g. of potassium hydroxide was allowed to stand at room temperature overnight. Dilution with water and extraction with ether removed a negligible amount of material, but ether extraction after acidification with hydrochloric acid furnished 0.85 g. of yellow oil which crystallized (0.61 g., m.p. 106–111°) after filtration through 30 g. of deactivated alumina. Repeated recrystallization from petroleum ether or from aqueous ethanol afforded yellow needles, m.p. 119–120°, gray-green color with ethanolic ferric chloride solution; $\lambda_{\max}^{\mathrm{CS_2}}$ 5.71(s), 5.83(s) μ, $\lambda_{\max}^{\mathrm{CHCl_3}}$ 5.77(s), 6.11(s) and 6.23(s) μ. The ultraviolet and visible absorption spectra (Fig. 2) showed $\lambda_{\max}^{\mathrm{EtOH-HCl}}$ 284 and 325 mμ (log ε 4.40, 3.96), $\lambda_{\min}^{\mathrm{EtOH-HCl}}$ 247 and 317 mμ (log ε 3.50, 3.94), $\lambda_{\max}^{\mathrm{EtOH-KOH}}$ 238, 298 and 415 mμ (log ε 4.20, 4.23, 4.23), $\lambda_{\min}^{\mathrm{EtOH-KOH}}$ 224, 265 and 335 mμ (log ε 4.10, 3.79, 3.35).

Anal. Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58; mol. wt., 372.4. Found: C, 70.86; H, 7.68; mol. wt., 376 (electrometric titration in 66% DMF, initial *p*H 3.5, pK_a' 5.2).

As was to be expected, saponification of mammein diacetate (II) (0.305 g.) with 30 ml. of 1 *N* methanolic potassium hydroxide overnight at room temperature yielded isomammein (VIII) rather than mammein (I). Mammein (I) however is stable to potassium carbonate in boiling acetone under the conditions used for the methylations.

Isomammein Dimethyl Ether (IX).—Anhydrous potassium carbonate (1.0 g.) and dimethyl sulfate (0.1 cc.) were added to a solution of isomammein (0.10 g.) in dry acetone (5 cc.). The mixture was stirred and refluxed for 1.5 hr. A small amount of potassium carbonate and dimethyl sulfate (0.1 cc.) then were added. Refluxing was continued for a total of 24 hr. The solution was filtered and the acetone evaporated giving a pale straw-colored oil (0.117 g.), which was dissolved in ether (10 cc.) and the ethereal solution washed with 2 *N* sodium hydroxide solution (10 cc.), twice with water, dried ($MgSO_4$) and evaporated giving a colorless oil (84 mg.). Chromatography on Merck acid-washed alumina gave by elution with 1:1 hexane–benzene, isomammein dimethyl ether as a colorless oil (61 mg.). The compound gave no color with ferric chloride and showed no titratable groups in 66% DMF; $\lambda_{\max}^{\mathrm{CHCl_3}}$ 5.80(s), 6.18(m) and 6.28(s) μ. The ultraviolet absorption spectrum showed $\lambda_{\max}^{\mathrm{EtOH}}$ 297 mμ (log ε 4.16) and a broad shoulder at 237–247 mμ (log ε 4.13). $\lambda_{\min}^{\mathrm{EtOH}}$ 268.5 mμ (log ε 3.96); it was not altered by the addition of alkali.

Anal. Calcd. for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05; O, 19.98; 2 OCH_3, 15.50. Found: C, 71.47; H, 7.88; O, 20.50; OCH_3, 15.90.

Isomammein Diacetate (X).—Acetylation with acetic anhydride in pyridine at room temperature overnight followed by recrystallization from aqueous isopropyl alcohol and from petroleum ether afforded colorless crystals of isomammein diacetate, m.p. 98–99°, depressed to 81–87° upon admixture with mammein diacetate (II). The ultraviolet absorption spectrum with $\lambda_{\max}^{\mathrm{EtOH}}$ 246 and 282 mμ (log ε 4.23 and 4.06) and $\lambda_{\min}^{\mathrm{EtOH}}$ 230 and 272 mμ (log ε 4.13 and 4.01) differed considerably from that of mammein diacetate (II).

Anal. Calcd. for $C_{26}H_{32}O_7$: C, 68.40; H, 7.07. Found: C, 68.36; H, 7.51.

Dihydroisomammein (XI). (a) By Isomerization of Dihydromammein (V).—A solution of 160 mg. of dihydromammein (V) in 25 cc. of methanol containing 1.4 g. of potassium hydroxide was left at room temperature overnight, diluted with water, acidified and extracted with ether. The resulting yellowish oil (130 mg.) crystallized from petroleum ether, m.p. 95–102°, and the melting point of the yellow crystals was raised to 122–124° after repeated recrystallization from dilute isopropyl alcohol, gray-green color with ethanolic ferric chloride solution; $\lambda_{\max}^{\mathrm{CHCl_3}}$ 5.78, 6.13 and 6.24 μ. The ultraviolet and visible absorption spectra (Fig. 2), $\lambda_{\max}^{\mathrm{EtOH-HCl}}$ 285 and 325 mμ (log ε 4.45, 4.05), $\lambda_{\min}^{\mathrm{EtOH-HCl}}$ 247 and 318 mμ (log ε 3.62, 4.04), $\lambda_{\max}^{\mathrm{EtOH-KOH}}$ 231.5, 298 and 415 mμ (log ε 4.30, 4.31, 4.16), $\lambda_{\min}^{\mathrm{EtOH-KOH}}$ 224, 264 and 335 mμ (log ε 4.29, 3.99, 3.59), resemble those of isomammein.

Anal. Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.68; H, 8.03.

(b) By Hydrogenation of Isomammein (VIII).—A solution of 290 mg. of isomammein in 20 cc. of methanol was shaken in an atmosphere of hydrogen with 50 mg. of platinum oxide catalyst and gas up-take stopped after one molar equivalent had been consumed. Filtration of the catalyst, dilution with water, extraction with ether and crystallization of the ether residue from dilute isopropyl alcohol furnished 260 mg. of dihydroisomammein (XI), m.p. 116–118°, raised to 121–123° on further recrystallization; identity was established by mixture melting point determination and infrared comparison.

Dihydroisomammein Dimethyl Ether (XII).—Methylation of dihydroisomammein (XI) with dimethyl sulfate and potassium carbonate in acetone solution was carried out as described above and after recrystallization from dilute isopropyl alcohol led to dihydroisomammein dimethyl ether (XII), m.p. 82–84°, depressed by 15° upon admixture with dihydromammein dimethyl ether (VII); $\lambda_{\max}^{\mathrm{CS_2}}$ 5.70, 5.82 and 6.17 μ, $\lambda_{\max}^{\mathrm{EtOH}}$ 246 and 297 mμ (log ε 4.08, 4.12); $\lambda_{\min}^{\mathrm{EtOH}}$ 238 and 269 mμ (log ε 4.06, 3.90).

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51; OCH_3, 15.43. Found: C, 71.59; H, 8.51; OCH_3, 15.50.

Ozonolysis of Mammein (I).—Ozone was passed at room temperature through a solution of 500 mg. of mammein in 10 cc. of glacial acetic acid until the exit gas showed the presence of ozone as indicated by moist starch iodide paper (30 min.). After stirring for one hour with 1 g. of ferrous sulfate in 15 cc. of water, the solution was steam distilled and the distillate (200 cc.) passed directly into a solution of 1.0 g. of 2,4-dinitrophenylhydrazine in 80 cc. of water and 20 cc. of concd. sulfuric acid. The resulting yellow 2,4-dinitrophenylhydrazone (163 mg., 51% yield) was purified by filtering in benzene solution through Merck acid-washed alumina and recrystallizing from hexane giving orange crystals, m.p. 125–126°, undepressed upon admixture with acetone 2,4-dinitrophenylhydrazone; the infrared spectra were also identical.

No evidence for the formation of other dinitrophenylhydrazones was obtained and under identical conditions a blank run did not furnish any detectable precipitate with the 2,4-dinitrophenylhydrazine reagent.

In a subsequent run with 927 mg. of mammein in 20 cc. of acetic acid[17] over a period of 78 minutes, the ozone consumption was measured quantitatively and found to equal 1.1 moles before ozone could be detected in the exit gases. The ozonide was decomposed by stirring for 1 hr. with 2.0 g. of ferrous sulfate in 20 cc. of water, but instead of steam distilling, 200 cc. of water was added and the product was extracted with ether. After washing with water and evaporating, there was obtained 868 mg. of the yellowish solid aldehyde XIV which was decolorized in ether solution with Norit. Chromatographic purification on Merck acid-washed alumina, elution with benzene–ether mixtures and recrystallization from aqueous methanol gave colorless crystals, m.p. 136–144°. The melting point was not improved on further recrystallization from ether–hexane or chloroform–hexane. The aldehyde gave a red-brown color with ferric chloride and in contrast to mammein showed a positive reaction with Tollens reagent; $\lambda_{\max}^{\mathrm{CS_2}}$ 3.0(w), 3.59(w) (not present in mammein), 5.70(w) and 5.82(m) μ; $\lambda_{\max}^{\mathrm{CHCl_3}}$ 3.01(w), 5.70(m), 5.80(s), 6.06(s) and 6.16(s) μ; $\lambda_{\max}^{\mathrm{EtOH}}$ 293 mμ (log ε 4.30), $\lambda_{\min}^{\mathrm{EtOH}}$ 249 mμ (log ε 3.50), $\lambda_{\max}^{1\,N\ \mathrm{EtOH-KOH}}$ 327 mμ (log ε 4.58) and shoulder at 380 mμ (log ε 4.29), $\lambda_{\min}^{1\,N\ \mathrm{EtOH-KOH}}$ 293 mμ (log ε 4.55).

Anal. Calcd. for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40; O, 27.72; mol. wt., 346. Found: C, 65.54; H, 6.66; O, 27.36; mol. wt., 351 (electrometric titration in 66% DMF, initial *p*H 5.9, *pK*$_a$′ 7.5).

A sample of mammein dimethyl ether (III) was ozonized in acetic acid solution as described above for mammein and yielded 35% of acetone 2,4-dinitrophenylhydrazone, uncontaminated by other dinitrophenylhydrazones. Treatment of dihydromammein (V) with ozone for 30 minutes showed that ozone could already be detected in the exit gases within 2–3 min. No steam-volatile products were formed and 45% of pure dihydromammein (V) could be recovered from the residue after ozonization and steam distillation.

Ozonization of Isomammein (VIII).—Since the ultraviolet spectral comparison (Figs. 1 and 2) between mammein (I) and isomammein (VIII) showed a marked change in the absorbing system, it was necessary to show by ozonization that the position of the reactive double bond was not altered in this transformation (I → VIII). Treatment of 498 mg. of isomammein (VIII) in 15 cc. of glacial acetic acid solution (yellow rather than colorless as with mammein) with ozone exactly as described above for mammein furnished 195 mg. (60%) of acetone 2,4-dinitrophenylhydrazone and 430 mg. of the crude aldehyde XV as a brownish solid. Decolorization with Norit in ether solution and recrystallization from ether–hexane gave transparent, pale yellow plates, m.p. 130–132°; $\lambda_{\max}^{\mathrm{CS_2}}$ 3.00(m), 3.60(w) (not present in isomammein), 5.70(m), 5.75(s), 5.81(s) μ; $\lambda_{\max}^{\mathrm{CHCl_3}}$ 3.02(m), 5.80(s), 6.15(s) μ; $\lambda_{\max}^{\mathrm{EtOH}}$ 281, 332 and 369 mμ (log ε 4.45, 4.07, 4.20), and a shoulder at 388 to 398 (log ε 4.14); $\lambda_{\min}^{\mathrm{EtOH}}$ 246 and 310 μ (log ε 3.81 and 4.01); $\lambda_{\max}^{1\,N\ \mathrm{EtOH-KOH}}$ 222.5, 305 and 408 mμ (log ε 4.56, 4.25, 4.24); $\lambda_{\min}^{1\,N\ \mathrm{EtOH-KOH}}$ 270, 340 and 371 mμ (log ε 4.12, 4.01, 4.11). While isomammein gives a gray-green color with ferric chloride and does not react with Tollens reagent, the aldehyde XV gave a green color and reduced Tollens reagent.

Anal. Calcd. for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40; O, 27.72; mol. wt., 346.4. Found: C, 65.40; H, 6.37; O, 28.05; mol. wt., 364 (electrometric titration in 66% DMF, initial *p*H 5.7, *pK*$_a$′ 7.6).

It was not possible to convert the aldehyde XIV, derived from mammein, by alkaline isomerization into the aldehyde XV, possibly due to other competing reactions since no crystalline product was isolated.

DETROIT, MICHIGAN
MAYAGUEZ, PUERTO RICO

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[17] The aldehyde XIV could not be isolated when the ozonization was conducted in ethyl acetate solution at −70°.

[CONTRIBUTION FROM CORN PRODUCTS REFINING COMPANY'S MULTIPLE FELLOWSHIP, MELLON INSTITUTE, AND THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF PITTSBURGH]

Polymerization of α-D-Glucose in the Solid State, in the Presence of Metaboric Acid[1]

BY HARRY W. DURAND,[2] MALCOLM F. DULL AND R. STUART TIPSON

RECEIVED NOVEMBER 16, 1957

The polymerization of crystalline α-D-glucose by heat in the presence of powdered metaboric acid has been studied. Optimal conditions for conducting the reaction are given, and the properties and evidence of the structural nature of typical polymeric products are described. One such product has been examined chromatographically and indicated to be a poly-D-glucoside of branched structure built up of both 1 → 6- and 1 → 4-acetal linkages.

Introduction

In 1945, Leuck[3] disclosed the successful polymerization of α-D-glucose in essentially the solid state, by heating of an intimate mixture of the crystalline anhydrous sugar with powdered boric acid or an anhydride thereof, in such a way that practically no fusion occurs. To the naked eye, the original shape of the sugar crystals is largely maintained throughout the reaction, but X-ray diffraction patterns show that the products are actually amorphous.

Leuck considered typical products to be polymeric on the basis of their infusibility, their lowered solubility in aqueous organic solvents, and the

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[1] Based on a thesis submitted by Harry W. Durand to the Graduate School of the University of Pittsburgh, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, February, 1956.

[2] Corn Products Refining Co., Argo, Ill.

[3] G. J. Leuck, U. S. Patent 2,375,564 (May 8, 1945); *C. A.*, **39**, 4508 (1945).