removed *in vacuo* and the reaction mixture poured into five volumes of water. The carbobenzoxy hexapeptide ester was extracted into ethyl acetate, which was washed with dilute hydrochloric acid, water, dilute sodium bicarbonate, water and dried over magnesium sulfate. Removal of the ethyl acetate *in vacuo* left a viscous oil, which was solidified by the addition of anhydrous ether; yield 7.7 g. (87%), softens at 174°, m.p. 175–178°, $[\alpha]^{25}D$ −96.0 (0.5% in methanol).

17. **H·Pro-Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe·HCl**(L-L-L-L-L-L).—7.5 g. (7.6 mmoles) of Z·Pro-Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe(L-L-L-L-L-L) (compound 16) was dissolved in 150 ml. of methanol containing 11.4 ml. of 1 *N* hydrochloric acid and hydrogenated over palladium black until carbon dioxide evolution ceased. The palladium was removed by filtration and the solvent distilled off *in vacuo*. The residual oil was made anhydrous by repeated additions of ethyl alcohol, followed by removal of the alcohol by distillation *in vacuo*. The product crystallized from methanol–ethyl acetate; yield 4.8 g. (72%), $[\alpha]^{26}D$ −81.0 (0.5% in 0.01 *N* hydrochloric acid).

NEW YORK, N. Y.

[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE]

The Constituents of *Ecballium elaterium* L. VI. The Functions of Elatericin A[1,2]

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The functions of the potent anti-tumor, naturally occurring compound elatericin A have been investigated. The following groupings were identified: an α,β-unsaturated ketone, an α-hydroxy-keto system easily autoxidizable with acid and alkali, and a third ketonic function probably in a hindered position. Elatericin A formed a diacetate, two acylatable hydroxyl groups being present. The molecule has been oxidized with periodic acid.

The isolation of elatericin A has been described in Part III of this series.[3] It occurs in the juice of the fruit of *Ecballium elaterium* and is obtained together with elatericin B by extraction with ether. The same compound has been isolated by Enslin[4a] from *Cucurbita pepo* and has been called by him Cucurbitacin D. This compound was also found in some other species of the Cucurbitaceae.[4]

Elatericin A, as well as some of the other constituents of this plant, has been found to have antitumor activity against Sarcoma 37[5] and Black Sarcoma in mice.[6]

The analyses of elatericin A consistently fitted the formula of $C_{28}H_{42}O_7$. Enslin, Rehm and Rivett[4b] suggested that the compounds of this series should be C_{30}-compounds unless an acetic acid ester group had to be attached to the molecule, the compounds being then of the C_{32}-type. The formula of $C_{30}H_{46}O_7 \cdot 0.5\ H_2O$ was therefore attributed to Cucurbitacin D. In view of recent developments on the structure of these compounds, we have adopted this formula, although it is only tentative and may be revised later.

Conclusions regarding the functional groups in elatericin A have been obtained from its spectroscopic data. The presence of an α,β-unsaturated ketone is indicated by a maximum in the ultraviolet light at 230 mμ (ϵ 10,000) (Fig. 1) and by bands at 1635 and 1689 cm.$^{-1}$ in the infrared. When elatericin A was hydrogenated over palladium, one mole of hydrogen was rapidly absorbed to form a dihydroelatericin A. In this compound the strong peak at 230 mμ had disappeared (Fig. 2) and no other strong absorption was found in ultraviolet. The double bond conjugated to the carbonyl group was therefore reduced. When alkali was added to a methanolic solution of elatericin A, an interesting observation was made. The strong peak at 230 mμ in the ultraviolet spectrum decreased rapidly, disappeared[4b] in three hours, and did not reappear upon acidification of the solution as shown in Fig. 1. By the action of alkali therefore, an irreversible change involving the α,β-unsaturated ketone takes place in the molecule. If the alkaline solution was allowed to stand a longer time it was observed that a new peak developed slowly in the ultraviolet at 310 mμ (ϵ 5,100) reaching its maximum 40 hours later.[4b] Acidification of the solution resulted in a shift of this peak to 268 mμ (ϵ 6,900); Fig. 1. The solution now gave a positive test with ferric chloride for a phenol group, and the infrared spectrum of the new product indicated two new bands at 1660 and 1413 cm.$^{-1}$, which were not seen in elatericin A; moreover a band at 1713 cm.$^{-1}$ originally present in that molecule had disappeared. It is noteworthy, that this change in the molecule occurring during addition of alkali was not reversible, and acidification did not restore the original molecule but shifted the new maximum to a shorter wave length. Compared to the rapid disappearance of the maximum at 230 mμ in the ultraviolet the rate of formation of the new peak at 310 mμ is very slow as shown in Fig. 3. There seems therefore to be no interrelationship between these two processes which must be independent; essentially dihydroelatericin A, whose ultraviolet spectrum did not show any maximum at 230 mμ, behaved in a similar way upon addition of alkali and a maximum gradually appeared at 310 mμ, Fig. 2.

The hypsochromic shift of about 52 mμ combined with an increase in intensity of absorption of about 30%, as well as the new infrared bands, clearly

(1) This investigation was supported by a research grant CY-2810(C) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) In this series of papers are to be considered Part IV, D. Lavie and Y. Shvo, *Proc. Chem. Soc.*, 220 (1958); and Part V, D. Lavie, Y. Shvo and D. Willner, *Chemistry & Industry*, 1361 (1958).

(3) D. Lavie and D. Willner, THIS JOURNAL, **80**, 710 (1958).

(4) (a) P. R. Enslin, *J. Sci. Fd. Agric.*, **5**, 410 (1954); (b) P. R. Enslin, S. Rehm and D. E. A. Rivett, *ibid.*, **8**, 673 (1957); (c) S. Rehm, P. R. Enslin, A. D. J. Meese and J. H. Wessels, *ibid.*, **8**, 679 (1957).

(5) D. Lavie, D. Willner, M. Belkin and W. G. Hardy, presented at the Symposium on the Chemotherapy of Cancer, Tokyo, October, 1957, abstracts, p. 53; *ACTA, Unio Int. Contra Cancrum*, in press.

(6) Unpublished data.

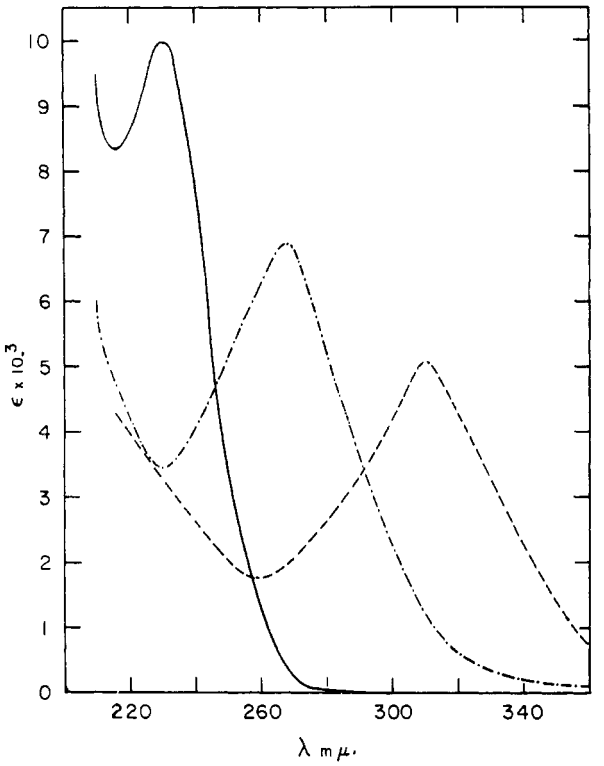


Fig. 1.—Ultraviolet absorption spectrum of elatericin A: ———, neutral solution in methanol; – – – –, 0.05 *N* NaOH in 10% aqueous methanol solution; –·–·–, alkaline solution acidified with HCl.

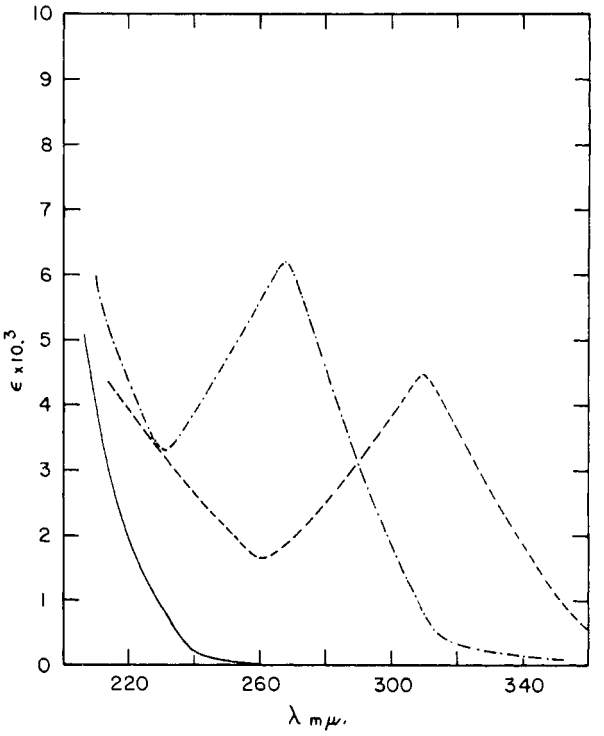


Fig. 2.—Ultraviolet absorption spectrum of dihydroelatericin A: ———, neutral solution in methanol; – – – –, 0.05 *N* NaOH in 10% aqueous methanol solution; –·–·–, alkaline solution acidified with HCl.

indicate the formation of a diosphenol chromophore.[7] We have previously assumed the formation of such a system by the slow enolisation of an α-diketone with alkali.[8] However, careful study of the different reactions involved during degradation studies which will be subsequently described, induced us to assume that the diosphenol system originated probably by autoxidation of an α-hydroxy-keto-system (I). Autoxidations of α-hydroxy-keto systems in alkaline or acidic solutions have often been described,[9] indeed adding acid to a solution of elatericin A also resulted in formation of the same chromophore. Oxidation of elatericin A with bismuth oxide in acetic acid resulted in a product having all the characteristics of a diosphenol,[3,7] and with triphenyltetrazolium chloride a red precipitate was formed.[10]

(7) D. Lavie and S. Szinai, THIS JOURNAL, **80**, 707 (1958).

(8) D. Lavie and Y. Shvo, *Proc. Chem. Soc.*, 220 (1958); *cf.* also ref. 4b.

(9) Cevine possesses a secondary ketol which is masked; in some preparations of cevine and on standing for several days in alkaline solutions the formation of a diosphenol chromophore has been observed; D. H. R. Barton and J. F. Eastham, *J. Chem. Soc.*, 424 (1953). These authors find that the formation of this chromophore was due to autoxidation. Dehydrocevagenin was reported by Auterhoff and Zeisner (ref. 10) to have a strong maximum in the ultraviolet at 270 mμ. In a model experiment we have added alkali to a solution of cevagenin and left the mixture for a few days. A slow forming maximum at 310 mμ was observed. This maximum shifted to 270 mμ when the solution was acidified, the intensity of absorption increasing thereby like in diosphenols. Dehydrocevagenin was therefore formed in the solution by autoxidation.

(10) H. Auterhoff and G. Zeisner, *Arch. der Pharm.*, **286** [58], 525 (1953).

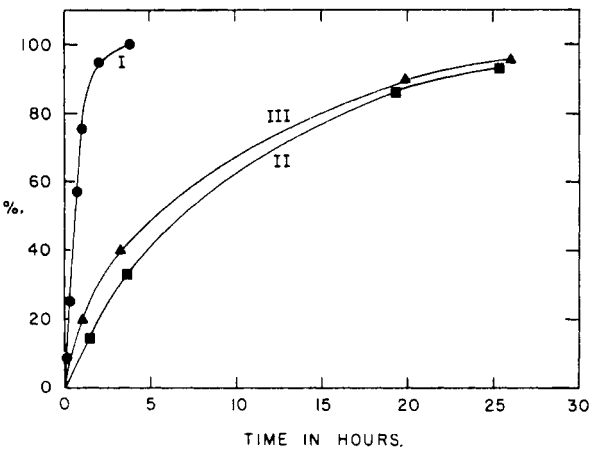
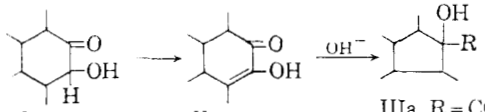


Fig. 3.—Rate of induced changes in ultraviolet by alkali in methanolic solution: I, rate of percentage disappearance of λ_{max} 230 mμ in elatericin A; II, rate of percentage appearance of λ_{max} 310 mμ in elatericin A; III, rate of percentage appearance of λ_{max} 310 mμ in dihydroelatericin A.

The easy formation of the α-diketone II resulted in reactions specific to this system; for instance, with *o*-phenylenediamine in the presence of acid a quinoxaline derivative could be obtained.[3] Heating elatericin A or its dihydro derivative in alkali resulted in the formation of an α-hydroxy-acid IIIa. The formation of such hydroxy-acids have been explained by a benzilic acid-like rearrangement.[7] However, in the case of elatericin A this rearrangement was much slower and the yields lower than for instance in α-elaterin, owing to the

necessary autoxidation involved in the reaction. Reduction of the methyl ester IIIb with lithium aluminum hydride resulted in a product devoid of



I II

IIIa, R = COOH
IIIb, R = $COOCH_3$
IIIc, R = CH_2OH

any carbonyl absorption; this product, which contained a newly formed glycol system IIIc, consumed two moles of periodic acid, while the methyl ester IIIb consumed one mole only of the acid. Distillation of the oxidation products of IIIc yielded formaldehyde, (identified as its dimedone derivative), thereby indicating the formation of the terminal primary alcohol vicinal to a hydroxyl group. The tertiary nature of this hydroxyl and the size of the ring to which it was connected were determined by a study of the infrared spectrum of the non-volatile amorphous oxidation products. It showed a strong and sharp band at 1738 cm.$^{-1}$ which is indicative for a ketone in a five-membered ring, thereby pointing toward a ring contraction occurring during the rearrangement.

Hydrogenation of elatericin A over palladium-on-charcoal resulted in the absorption of one mole of hydrogen; no more than one mole of hydrogen could be introduced with this catalyst. In this product, dihydroelatericin A, according to spectroscopic evidences, the conjugated double bond was reduced. As mentioned earlier, the dihydro derivative with alkali also formed a diosphenol; Fig. 2. Elatericin A as well as the dihydro derivative gave bis-2,4-dinitrophenylhydrazones, two carbonyl groups reacting with the reagent. The infrared spectra of each of these derivatives showed a band ν_{max} 1690 cm.$^{-1}$ for a ketone; it can therefore be assumed that a third, probably unreactive carbonyl, is present in the molecule. During hydrogenation of dihydroelatericin A over platinum, a second mole of hydrogen was absorbed, and tetrahydroelatericin A was obtained. From the three carbonyl groups known to be in the molecule, one was expected to be reduced; however, the spectroscopic indications were different. A careful study of the infrared spectrum of the carbonyl region using a calcium fluoride prism indicated that only one absorption remained at 1693 cm.$^{-1}$. Moreover the new derivative did not react at all with 2,4-dinitrophenylhydrazine, and the ultraviolet spectrum in neutral as well as in alkaline solution did not show any absorption. Table I shows the absorption frequencies in the carbonyl region of the different compounds using a calcium fluoride prism, which allows a detailed study of the character of the different carbonyl functions.

In elatericin A the band at 1689 cm.$^{-1}$ is related to the α,β-unsaturated carbonyl. Reduction of the double bond resulted in a shift of this absorption to 1705 cm.$^{-1}$. The 1696 cm.$^{-1}$ frequency is related to a ketone located in a hindered position, which is unreactive and does not form a 2,4-dinitrophenylhydrazone derivative. This ketone could not be hydrogenated and was reduced by boiling with lithium aluminum hydride. The third carbonyl of the molecule absorbed at 1712 cm.$^{-1}$, and is probably related to the α-hydroxy-keto system previously described. The disappearance of two carbonyl frequencies during the formation of the tetrahydro derivative, following the absorption of only one mole of hydrogen, can best be explained by the formation of a hemiketal between the newly formed alcoholic hydroxyl and a carbonyl group available for such a linkage. This would leave in the molecule the carbonyl group absorbing at 1693 cm.$^{-1}$. By allowing the hydrogenation to continue until two moles of hydrogen was absorbed a hexahydro derivative was formed. This derivative had only one carbonyl absorption frequency ν_{max} 1698 cm.$^{-1}$.

TABLE I

SUMMARY OF INFRARED ABSORPTION SPECTRA[a]

Compound	Frequencies, cm.$^{-1}$				
Elatericin A	1635	1689	1696		1712
Dihydroelatericin A			1698	1705	1713
Tetrahydroelatericin A			1693		
Hexahydroelatericin A			1698		

[a] Measurements were done in a Perkin–Elmer single-beam spectrometer using a calcium fluoride prism and in chloroform solution.

Acetylation at room temperature of elatericin A yielded a diacetate. That neither of these acetoxy groups was enolic was shown by the ultraviolet and infrared spectra. The latter still indicated the presence of at least one non-acylatable hydroxyl group (ν_{max} 3420 cm.$^{-1}$), probably tertiary. Acetylation under the same conditions, of the product obtained from the cold alkaline treatment of elatericin A, containing the diosphenol system, yielded an amorphous acetylation product, one of the acetoxy groups being enolic as was indicated by the spectroscopic data: λ_{max} 233 mμ.

Summing up the different groupings present in the molecule of elatericin A, there are three carbonyl groups one of which is an α,β-unsaturated ketone, two acylatable hydroxyls and at least one tertiary non-acylatable hydroxyl. The nature of the last oxygen is still undetermined.

The many oxygen functions in elatericin A and its derivatives invite inquiry into their susceptibility to attack by periodic acid. Table II summarizes the results of these experiments.

TABLE II

Compound	Uptake of HIO_4, moles, in 24 hours
Elatericin A	1.91
Elatericin A acetate	0.21
Dihydroelatericin A	2.11
Tetrahydroelatericin A	1.95
Hexahydroelatericin A	1.85

A detailed study of the products of the periodic acid oxidation, indicating the relationship between the different oxygen functions, will be described in the following paper.

Acknowledgment.—The authors are indebted to Dr. S. Pinchas for the determination of the infrared spectra with calcium fluoride prism and for contributing to their interpretation. Thanks are

due to Mrs. R. Tugenhaft for technical assistance and to Mr. E. Meier for the microanalyses.

Experimental

All melting points are uncorrected.

Spectroscopic Measurements.—Ultraviolet absorption spectra were done on a Unicam model S.P. 500 spectrophotometer. Infrared spectra were obtained on a Baird double-beam spectrometer equipped with a sodium chloride prism or, when specified, on a Perkin–Elmer single-beam model 12C spectrometer equipped with a calcium fluoride prism. Unless otherwise stated, all spectra were determined in chloroform solutions of 50 mg. per ml. concentration.

Isolation of Elatericin A.—Elatericin A was isolated from the juice of the fruit of *Ecballium elaterium* by continuous extraction with ether as described in paper III[3] of this series. Elatericin A was separated from elatericin B using the chemical method described there. The product was crystallized several times from equal parts of ethyl acetate–benzene; white needles, m.p. 151–153°, $[\alpha]_D$ + 48° in chf. (*c* 1.5); ν_{max} 3425, 1712, 1696, 1689 and 1635 cm.$^{-1}$ (calcium fluoride prism); λ_{max} 230 mμ (ε 10,000) in ethanol; with alkali, 0.05 *N* NaOH in 10% aqueous methanolic solution, after 3 hours λ_{max} 230 mμ disappeared; after 40 hours λ_{max} 310 mμ (ε 5,100); upon acidification of this alkaline solution with hydrochloric acid λ_{max} 268 mμ (ε 6,900). No coloration was observed with ferric chloride; a red precipitate was formed with triphenyltetrazolium chloride.[10]

Anal. Calcd. for $C_{30}H_{46}O_7 \cdot 0.5H_2O$: C, 68.28; H, 8.98; CH_3C, 14.2. Found: C, 68.50; H, 8.72; CH_3C, 13.1.

Bis-2,4-dinitrophenylhydrazone, orange crystals from butanol, m.p. 218–220° dec., ν_{max}^{KBr} 1690 cm.$^{-1}$, λ_{max} 367 mμ (ε 42,000) in ethanol.

Anal. Calcd. for $C_{42}H_{54}O_{13}N_8 \cdot H_2O$: C, 56.23; H, 6.29; N, 12.49. Found: C, 56.20; H, 6.11; N, 13.19.

Elatericin A Diacetate.—Elatericin A (250 mg.) was acetylated in a mixture of acetic anhydride (0.8 ml.) and pyridine (2.5 ml.) by heating on a steam-bath for one hour. The solution was decomposed with ice-water and the amorphous residue collected by filtration (210 mg.). Repeated crystallizations from benzene–petroleum ether yielded rectangular plates, m.p. 184–186°, $[\alpha]_D$ −19° (*c* 1.21) in chf.; ν_{max} 3430, 1730, 1695 and 1630 cm.$^{-1}$; λ_{max} 230 mμ (ε 10,000).

Anal. Calcd. for $C_{34}H_{50}O_9$; C, 67.75; H, 8.36; CH_3CO, 14.3. Found: C, 67.98; H, 8.22; CH_3CO, 14.6.

Dihydroelatericin A.—Elatericin A (250 mg.) in ethanol was hydrogenated over palladium-on-charcoal (5%, 80 mg.). The absorption of hydrogen ceased when 0.9 mole was consumed (5 minutes). The catalyst was filtered and the solvent evaporated under reduced pressure. The residue crystallized from ethyl acetate–petroleum ether, microcrystals, m.p. 168° (clear melt), $[\alpha]_D$ + 83° (*c* 1.27) in ethanol; ν_{max} 3430, 1713, 1705 and 1698 cm.$^{-1}$ (calcium fluoride prism); the ultraviolet absorption spectrum in neutral solution did not show any maximum; with alkali, 0.05 *N* NaOH in 10% aqueous methanolic solution, after 40 hours, λ_{max} 310 mμ (ε 4,500); upon acidification of this alkaline solution with hydrochloric acid, λ_{max} 268 mμ (ε 6,200). No coloration with ferric chloride; a red precipitate was formed with triphenyl tetrazolium chloride.

Anal. Calcd. for $C_{30}H_{48}O_7$: C, 69.20; H, 9.29. Found: C, 69.20; H, 8.97.

The product crystallized from diluted ethanol as needles, m.p. 143–145°.

Anal. Calcd. for $C_{30}H_{48}O_7 \cdot H_2O$: C, 66.88; H, 9.36. Found: C, 67.19; H, 9.17.

Bis-2,4-dinitrophenylhydrazone, yellow crystals from butanol, m.p. 215–218° dec., ν_{max}^{KBr} 1690 cm.$^{-1}$, λ_{max} 365 mμ (ε 38,000) in ethanol.

Anal. Calcd. for $C_{42}H_{56}O_{13}N_8$: C, 57.26; H, 6.40; N, 12.72. Found: C, 57.03; H, 6.70; N, 12.73.

Tetrahydroelatericin A.—Elatericin A (410 mg.) in ethanol (25 ml.) was hydrogenated over platinum. The hydrogenation was discontinued after two moles of hydrogen was absorbed (10 minutes). The catalyst was filtered and the solution evaporated under reduced pressure. The residue crystallizes from dilute ethanol, silky needles (200 mg.), m.p. 152–156°, $[\alpha]_D$ +31° (*c* 1.3) in ethanol, ν_{max} 3430 and 1693 cm.$^{-1}$ (calcium fluoride prism); the ultraviolet spectrum did not show any maximum absorption in neutral or in alkaline solution; no coloration with ferric chloride nor with triphenyl tetrazolium chloride. The product did not react to form a 2,4-dinitrophenylhydrazone.

Anal. Calcd. for $C_{30}H_{50}O_7 \cdot 3H_2O$: C, 62.47; H, 9.79. Found: C, 62.70; H, 9.67.

Hexahydroelatericin A.—Elatericin A (345 mg.) in ethanol (25 ml.) was hydrogenated over platinum (80 mg.). The calculated amount for three moles of hydrogen was absorbed during one hour. The catalyst was filtered and the solvent evaporated under reduced pressure. Needles from nitromethane (150 mg.), m.p. 168° (clear melt), $[\alpha]_D$ +70° (*c* 1.25) in ethanol, ν_{max} 3425 and 1693 cm.$^{-1}$ (calcium fluoride prism); the ultraviolet spectrum did not show any maximum absorption in neutral or in alkaline solution; no coloration with ferric chloride nor with triphenyltetrazolium chloride. The product did not react to form a 2,4-dinitrophenylhydrazone. No consistent analyses could be obtained.

Rearrangement of Dihydroelatericin A.—Dihydroelatericin A (800 mg.) in ethanol (25 ml.) was added to an 8% solution (25 ml.) of sodium hydroxide and heated under nitrogen for 4 hours. Part of the ethanol was evaporated under reduced pressure and water (25 ml.) was added. The mixture was then extracted several times with ether to separate unreacted material and the solution acidified with hydrochloric acid. The amorphous acid formed was extracted with ethyl acetate and dried over sodium sulfate. Evaporation of the solvent left a residue (300 mg.) of a white microcrystalline powder. This powder was further purified by dissolving in sodium bicarbonate and reprecipitated with acid; decomposed between 126–133° (IIIa).

The methyl ester IIIb of this acid was prepared with a solution of diazomethane in ether. When the gas evolution subsided, the solvent was evaporated. The residue was redissolved in ether and shaken with a solution of bicarbonate to eliminate unreacted acid, the ethereal fraction was separated dried over sodium sulfate and the solvent evaporated. White amorphous powder which could not be induced to crystallize.

Reduction of IIIb to IIIc.—A solution of the methyl ester IIIb (480 mg.) in dry ether (50 ml.) was added slowly to a stirred solution of lithium aluminum hydride (1 g.) in ether (100 ml.). The mixture was heated and stirred for 60 hr., then hydrolyzed with dilute mineral acid. The ether layer was separated and the aqueous fraction continuously extracted with ether for 24 hours. This ether extract was dried over sodium sulfate and evaporated; the residue crystallized from nitromethane, needles, m.p. 130–132° (110 mg.) of IIIc, ν_{max}^{KBr} 3450 (s) cm.$^{-1}$.

Periodic Acid Oxidation of IIIc.—Periodic acid (260 mg.) in 2 ml. of water was added to a solution of IIIc (180 mg.) in methanol (5 ml.) and left overnight at room temperature. The reaction mixture was then distilled *in vacuo* with a bath temperature of 50–60° into a solution of methone. The crystalline material obtained from the distillate was filtered, needles (20 mg.), m.p. 189–191°. Mixture m.p. with an authentic sample of formal dimedone was not depressed.

Formaldehyde was therefore formed during the oxidation. The residue left after the distillation was taken up with ether, washed with water, dried over sodium sulfate and evaporated to dryness; oily material (50 mg.); ν_{max} 3450, 1738 (cyclopentanone) and 1700 cm.$^{-1}$.

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