Anal. Calcd. for $C_{27}H_{46}O_7$: C, 68.06; H, 8.40. Found: C, 68.21; H, 8.24.

Reduction of Methyl Ecballate (III).—A solution of methyl ecballate (450 mg.) in dry ether (100 ml.) was added slowly to a solution of lithium aluminum hydride (1 g.) in dry ether (200 ml.) with stirring. The mixture was heated under reflux for 60 hours, stirring being continued. The reaction mixture was decomposed with ice and dilute hydrochloric acid was added. The aqueous layer was separated and extracted continuously with chloroform for 24 hours. Distillation of the solvent yielded a white powder (350 mg.). This powder was triturated with very dilute aqueous ethanol and the microcrystalline product (200 mg.) was air-dried and washed with ether, m.p. 149–150° dec., ν_{max}^{Nujol} 3350 cm.$^{-1}$ (strong).

Periodic Acid Oxidation of III.—Periodic acid (100 mg.) in 3 ml. of water was added to a solution of the reduced product III (50 mg.) in ethanol (5 ml.), and the mixture was allowed to stand overnight. Water was added and the reaction product extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate and distilled. The amorphous residue could not be crystallized (35 mg.), ν_{max} 1737 cm.$^{-1}$ (cyclopentanone).

Elaterin Methyl Ether.—A mixture of elaterin (1 g.), freshly dried anhydrous potassium carbonate (3 g.) and methyl iodide (5 g.) was heated to reflux in acetone (30 ml.) under nitrogen with stirring for three days.[18] During this time two portions of methyl iodide (5 g.) were added at 24-hour intervals. A negative ferric chloride test of the reaction mixture indicated the end of the reaction. The solution was filtered and the salts washed with acetone. The combined filtrate and washings were evaporated and the residue (0.9 g.) crystallized from ethanol–water; microcrystals, m.p. 116–118°; ν_{max} 3450, 1720, 1680, 1625 and 1370 cm.$^{-1}$. An analytical sample was dried to constant weight at 77°.

Anal. Calcd. for $C_{33}H_{46}O_5 \cdot H_2O$: C, 67.32; H, 8.22. Found: C, 67.46; H, 8.17.

Elaterin Diacetate.—Elaterin (1 g.) was acetylated in boiling acetic anhydride (25 ml.) for two hours. The mixture was decomposed with ice-water and the product dissolved in ether. The ether solution was washed with water, dried over sodium sulfate and evaporated. The residue (0.9 g.) was triturated with dilute ethanol (excess water) and the microcrystalline product filtered and recrystallized twice from ethanol–water; colorless microcrystals, m.p. 124–126°; ν_{max} 3440, 1737, 1694, 1630, 1368, 1082, 1027 and 994 cm.$^{-1}$.

Anal. Calcd. for $C_{36}H_{48}O_{10} \cdot 0.5H_2O$: C, 66.62; H, 7.61; CH_3CO, 19.84. Found: C, 66.31; H, 7.78; CH_3CO, 20.01.

Elateridin Diacetate.—A solution of elateridin (500 mg.) in dry pyridine (10 ml.) and acetic anhydride (10 ml.) was left overnight at room temperature. The mixture was poured into ice-water and the oily product extracted with ether. The ether solution was washed with water, dried and evaporated. The residue (450 mg.) was triturated with a mixture of ethanol and water. The microcrystalline substance obtained was filtered and recrystallized three times from ethanol–water, using an excess of water. At this stage the melting point was constant: m.p. 136–138°.

The same product was obtained when the acetylation was carried on in boiling acetic anhydride for three hours.

Anal. Calcd. for $C_{34}H_{46}O_9$: C, 68.28; H, 7.75; CH_3CO, 14.37. Found: C, 68.46; H, 8.09; CH_3CO, 14.38.

Pyroelaterin.—Elaterin (437 mg.) was heated under nitrogen to 260°, and the colorless liquid which distilled was collected in a cold trap (46 mg.). This distillate had the characteristic pungent smell of acetic acid; b.p. 116–118°, *p*-bromophenacyl ester[20] m.p. 85.5°. A mixture m.p. with an authentic sample showed no depression. The calculated amount for one mole of acetic acid is 47 mg. The crude melt was collected and a sample ground and analyzed.

Anal. Calcd. for $C_{30}H_{40}O_6$: C, 72.58; H, 8.06. Found: C, 71.86; H, 8.17.

Good results were obtained when the pyrolysis was carried out at reduced pressure.

The melt was dissolved in methanol, water was added until the mixture became cloudy, and left to stand overnight, giving white microcrystals which were recrystallized several times from the same solvents mixture; m.p. 292–294°, $[\alpha]_D$ −21° in chf. (*c* 0.28); coloration with ferric chloride; positive iodine test with potassium iodide solution in acetic acid.[21]

Anal. Calcd. for $C_{30}H_{40}O_8$: C, 68.16; H, 7.63. Found: C, 68.30; H, 7.51.

Bis-2,4-dinitrophenylhydrazone, yellow microcrystals from ethanol–water, m.p. 285–287°.

Anal. Calcd. for $C_{42}H_{48}O_{14}N_8$: C, 56.8; H, 5.5; N, 9.7. Found: C, 56.8; H, 5.8; N, 10.0.

Dioxime, crystals from ethanol–water, m.p. 219–221° (hot-stage).

Anal. Calcd. for $C_{30}H_{46}O_8N_2$: C, 64.05; H, 8.18; N, 4.98. Found: C, 64.31; H, 8.13; N, 5.14.

(20) C. G. Moses and E. E. Reid, THIS JOURNAL, **54**, 2101 (1932).

(21) Houben-Weyl, "Methoden der Organischen Chemie," Sauerstoffverbindungen Vol. III, Georg Thieme Verlag, Stuttgart, 1952, p. 63.

REHOVOTH, ISRAEL

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

The Constituents of *Ecballium elaterium* L. III. Elatericin A and B[1,2]

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Elatericin A and B, two new compounds with anti-tumor activity, have been isolated from the fruit of *Ecballium elaterium* L. The oxygen functions of elatericin B have been determined by ultraviolet and infrared spectroscopy, hydrogenation, acetylation and methylation. By treatment with alkali, an acid was obtained which was further degraded.

In part II,[3] the isolation of elaterium from the juice of fruits of *Ecballium elaterium* was described. When the clear supernatant liquid was continuously extracted with ether, a mixture of amorphous bitter principles was obtained. This mixture showed strong anti-tumor activity against Sarcoma 37 in mice.[4] Belkin and Fitzgerald,[5] who investigated plants having cathartic properties as possible anti-neoplastic agents, found that elaterium was among the most potent. Now it has been found that such activity was also present in the amorphous ether extract of fresh juice and in the crys-

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

(2) Presented in part before the XIX Meeting of the Israel Chemical Society, Rehovoth, June, 1956; *Bull. Res. Counc. of Israel*, **5A**, 284 (1956).

(3) Part II, D. Lavie and S. Szinai, THIS JOURNAL, **80**, 707 (1958).

(4) The bio-assays were made by M. Belkin and W. Hardy at The National Cancer Institute, Bethesda, Md., and will be published elsewhere.

(5) M. Belkin and D. B. Fitzgerald, *J. Nat. Cancer Inst.*, **13**, 139, (1952).

talline compounds isolated from this extract. Recently Enslin[6] and Enslin and Rivett[7] have investigated the bitter principles which they have found to occur in many *Cucurbitaceae* and have designated them cucurbitacins. Steyn[8] has reported human poisoning caused by bitter principles of cultivated varieties of pumpkins, squashes and watermelons, in these cases the bitterness originating from intercrossings with wild varieties and mutations.[9a]

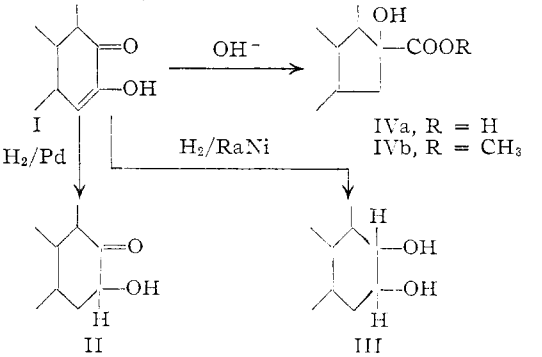
When the amorphous extract which was obtained from the juice of *Ecballium* was crystallized from ethyl–acetate benzene, a white crystalline product was obtained. Chromatography on paper impregnated with formamide followed by spraying with a solution of potassium permanganate and copper acetate, a method developed by Enslin, Joubert and Rehm,[9] showed two blue spots. These two spots could also be developed when the paper was put in an atmosphere of iodine vapors.[10] Good separation of substantial amounts of the two substances was obtained by chromatography through a silicic acid column using a 50% ethyl acetate–benzene mixture equilibrated with formamide for elution. The two substances which were found to be homogeneous and which had the highest anti-tumor activity of all the fractions tested were called, respectively, elatericin A and B.[11]

Elatericin B showed a strong positive ferric chloride test for phenols and was soluble in dilute aqueous alkali; this solubility behavior was subsequently used for separation of larger amounts of elatericin A and B. This paper will deal with elatericin B. The ultraviolet spectrum of elatericin B shows a major peak at 234 mμ (ϵ 11,000) and a shoulder at 266 mμ (ϵ 6,850); in alkaline solution the second maximum suffers a reversible bathochromic shift to 314 mμ (ϵ 6,750) due to formation of an enolate. As with elaterin,[3] a diosphenol system and an α,β-unsaturated ketone could be indicated. Infrared spectrum showed bands at 1685 (conjugated ketone) and 1629 cm.$^{-1}$ (conjugated double bond), and at 1660 and 1413 cm.$^{-1}$ for the diosphenol chromophore. The presence of a diosphenol system was substantiated by formation of a quinoxaline derivative with *o*-phenylenediamine (ultraviolet spectrum showed characteristic absorption at 239 and 320 mμ). For comparison we measured the spectrum of cyclohexanedione in the carbonyl region. Bands observed at 1660 and 1721 cm.$^{-1}$ showed that cyclohexanedione in chloroform occurs as a mixture of the diketo form (higher frequency) and the keto–enol form (lower frequency). On the other hand, Le Fevre, *et al.*, and others[12] found from spectroscopic data that diosphenol (*i.e.*, buchucamphor) occurs only in the keto–enol form. This is evidently the case in elatericin B, no carbonyl frequency higher than 1685 cm.$^{-1}$ appearing. Our ultraviolet data are also in reasonable accord with those reported for diosphenol.[12]

Heating elatericin B to reflux with aqueous alkali induced a benzilic acid-like rearrangement of the diosphenol grouping. The α-hydroxy acid IVa thus formed, called elatericinic acid, was found by analysis and by titration with tetrabutylammonium hydroxide as titrant in anhydrous pyridine to have an empirical formula of $C_{28}H_{44}O_8$. Elatericinic acid was further degraded by the same sequence used for elaterin.[3] In this way methyl elatericinate (IVb) was converted with lithium aluminum hydride to an amorphous substance, elatericinol, having a glycol system which was split with periodic acid to a carbonyl compound, norelatericinone, containing a five-membered cyclic ketone (1733 cm.$^{-1}$ band in the infrared). A second band at 1698 cm.$^{-1}$ also appeared for which no explanation can be given at this stage. Unlike ecballic acid[3] obtained from elaterin, elatericinic acid gave a negative iodoform test.[13]

When elatericin B was reduced with palladium-on-charcoal, one mole of hydrogen was rapidly absorbed. The hydrogenation product, dihydroelatericin B, no longer possessed a double bond conjugated to ketone inasmuch as the peak at 234 mμ had disappeared. The new ketone showed a band at 1691 cm.$^{-1}$. Although this is a low frequency for a saturated ketone, it is in agreement with observations made on ketones which are in a hindered position.[14]

By allowing two moles of hydrogen to be absorbed, a tetrahydro derivative was obtained; no more than two moles of hydrogen could be introduced with palladium-on-charcoal. The second mole of hydrogen had reduced enol I to ketol II in which the properties associated with the diosphenol system had disappeared, *i.e.*, the ferric chloride test was negative and the compound was insoluble in alkali. The newly formed saturated ketone II in addition to the 1694 cm.$^{-1}$ peak showed a new band in the infrared at 1710 cm.$^{-1}$. When elatericin B was reduced with Raney nickel and hydrogen under pressure, three moles was absorbed and the glycol III obtained.



(6) P. R. Enslin, *J. Sci. Fd. Agric.*, **5**, 410 (1954).

(7) P. R. Enslin and D. E. A. Rivett, *J. Chem. Soc.*, 3682 (1956).

(8) D. G. Steyn, *S. Afr. med. J.*, **24**, 713 (1950).

(9) (a) P. R. Enslin, T. G. Joubert and S. Rehm, *J. S. afr. Chem. Inst.*, **7**, 131 (1954); (b) R. B. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, **188**, 763 (1951).

(10) G. B. Marini-Bettolo, *Rendiconti Dell Instituto Superiore di Sanita*, **17**, 471 (1954).

(11) Private communication: Dr. Enslin kindly informed us that elatericin A and B were found to be identical with cucurbitacin D and I.

(12) R. J. W. Le Fevre, F. Maramba and R. L. Werner, *J. Chem. Soc.*, 2496 (1953); G. Schwarzenbach and C. Wittwer, *Helv. Chim. Acta*, **30**, 663 (1947); H. S. French and M. E. T. Holden, THIS JOURNAL, **67**, 1239 (1945).

(13) D. E. A. Rivett and F. H. Herbstein, *Chemistry & Industry*, 393 (1957).

(14) A. R. H. Cole and D. W. Thornton, *J. Chem. Soc.*, 1007 (1956).

The glycol still showed the 1694 cm.$^{-1}$ carbonyl absorption. The assumption that this absorption is due to a saturated, chemically hindered ketone found support in the fact that this ketone could be reduced only under very drastic conditions. Heating with lithium aluminum hydride and ether for 27 hours left the compound unchanged, and only through a modification of a Wolff–Kishner reduction procedure developed by Barton, Ives and Thomas[15] for sterically hindered carbonyl groups could an amorphous substance in which this ketone had been reduced be obtained. Interestingly, when methyl elatericinate (IVb) was reduced to elatericinol with lithium aluminum hydride in hot ether for 60 hours, reduction of this ketone did take place.

Acetylation of elatericin B with acetic anhydride at room temperature formed a crystalline diacetate. In this diacetate the enol grouping as in I was left unchanged (positive ferric chloride test and band at 3400 cm.$^{-1}$ for phenolic hydroxyl). In boiling acetic anhydride containing fused sodium acetate, a triacetate was formed. Here the enol was acetylated, as shown by an absorption (shoulder) at 1750 cm.$^{-1}$. The presence of a non-acylable hydroxyl was indicated by a band at 3510 cm.$^{-1}$. When the enol I of elatericin B was methylated with methyl iodide and potassium carbonate[16] before acetylation, elatericin B methyl ether was formed. The ultraviolet spectrum of this compound showed only one peak at 231 mμ, and in alkaline solution no shift to longer wave length was observed. On acetylation, a diacetate methyl ether was obtained containing a free hydroxyl group (absorption at 3510 cm.$^{-1}$).

A non-acylated hydroxyl group also was found in the tetraacetate obtained in the acetylation of the glycol III. These observations characterize the functions of three oxygens in addition to the three in the ketone and diosphenol groupings. Two of the three oxygens are secondary or primary alcohols, the third is a non-acylatable hydroxyl, presumably tertiary.

The last of the seven oxygen atoms present in elatericin B is probably etheric in nature. It is assumed that this oxygen is part of a furan ring on the following grounds: all derivatives in this series showed a band at 1095 cm.$^{-1}$; in the hydrogenated derivative an absorption was found in the ultraviolet as low as 212 mμ. Such a low absorption was also found in marrubin[17] (208, 212 and 216 mμ) and in columbin[18] (210 mμ) both of which have been shown to contain a furan ring, and is in substantial agreement with the absorption expected of a substituted furan.[19] The infrared spectrum of elatericin B also showed many bands in common with marrubin and columbin at frequencies regarded as characteristic for the furan ring (*e.g.*, 1005, 1090, 1606 cm.$^{-1}$). Further support for the presence of a furan system was obtained by a peculiar behavior observed during ultraviolet absorption measurements. When a drop of alkali was added to the alcoholic solution of the product, a new and strong absorption appeared at 273 mμ. This new absorption did not disappear on acidification, and reached its maximum within one hour. Many furan derivatives which we exposed to the same conditions exhibited similar behavior. An account of these experiments will be published later.

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Experimental

All melting points reported are uncorrected.

Spectrophotometric Measurements.—Ultraviolet absorption spectra were done on a Beckman model DU quartz spectrophotometer in ethanol solution; special measurements 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. Unless otherwise stated all spectra were determined in chloroform solutions of 50 mg. per ml. concentration. We are indebted to Mr. Erich Meier for the microanalyses.

Isolation of Elatericin A and B.—Decanted juice (40 liters) of fruit of *Ecballium elaterium* was continuously extracted with ether for 7 days. The extraction was made in two extractors of a capacity of 20 liters each, the ether being changed once after 3 days. The combined ether extracts (4 liters) were washed with dilute sodium bicarbonate and several times with water. The ether layer was dried over anhydrous sodium sulfate and evaporated. The residue was then treated under vacuum with slight heating on a water-bath until an amorphous yellow powder was obtained (50 g.). This powder was dissolved in a small quantity of ethyl acetate, and an equal amount of benzene added; white needles (22.3 g.) of a mixture of elatericin A and B, yield, calculated on fresh juice, 0.37%. This yield ranged in different batches from 0.3 to 0.5%.

Chromatography was made on paper Whatman No. 1 impregnated with formamide, 25% solution in alcohol, and developed with chloroform using the descending method. The chromatograms were dried in a current of hot air and then sprayed with a 0.5% solution of potassium permanganate in a saturated aqueous solution of copper acetate.[9] When heated a few minutes in an oven at 100°, two blue spots appeared, elatericin B having the highest R_f value. When the developed chromatogram was put in a jar in an atmosphere of iodine vapors, two brown spots were obtained.

Separation of Elatericin A and B. a. Chromatographic Method.—A column was packed with a mixture of silicic acid, 100 mesh for chromatography (80 g.),[20] and Celite (30 g.). A mixture of equal quantities of ethyl acetate and benzene was equilibrated with freshly distilled formamide and passed through the column. The crystalline mixture (1 g.) was then dissolved in the same solvents mixture and introduced into the column. Development was also made with the same solvents. From the 29 fractions collected (30 ml. each) those showing only one spot on the paper chromatogram were combined; by this way fractions 6 to 11 and 14 to 29 were reunited and separately crystallized a few times from ethyl acetate–benzene, the first one yielding **elatericin B**, needles (0.22 g.), m.p. 148–149° dec., $[\alpha]_D$ −52° in chf. (*c* 1.56); ν_{max} 3410, 1685, 1660, 1629, 1606, 1413, 1090 and 1005 cm.$^{-1}$; λ_{max} 234 mμ (ϵ 11,000), 266 mμ (ϵ 6,850); with alkali: λ_{max} 234 mμ (ϵ 11,000), 314 mμ (ϵ 6,750). In ethanol it gave a strong coloration with ferric chloride.

(15) D. H. R. Barton, D. A. J. Ives and B. R. Thomas, *J. Chem. Soc.*, 2056 (1955).

(16) F. H. Curd and A. Robertson, *ibid.*, 437 (1933); B. A. Hems and A. R. Todd, *ibid.*, 1208 (1940).

(17) W. Cocker, B. E. Cross, S. R. Duff, J. T. Edward and T. F. Holley, *ibid.*, 2540 (1953).

(18) D. H. R. Barton and D. Elad, *ibid.*, 2085 (1956).

(19) W. C. Price and A. D. Walsh, *Proc. Roy. Soc.* (*London*), **A179**, 201 (1941).

(20) Mallinckrodt Chemical Works, prepared by the method of Ramsey and Patterson.

Anal. Calcd. for $C_{28}H_{42}O_7$: C, 68.54; H, 8.63. Found: C, 68.53; H, 8.46.

Elatericin A, was obtained from fractions 14–29, needles (0.55 g.), m.p. 150–152° dec., $[\alpha]_D$ +46° in chf. (*c* 1.40), ν_{max} 3425, 1689, 1626, 1377, 1088, 1058 and 983 cm.$^{-1}$; λ_{max} 232 mμ (ϵ 9,000). In ethanol no coloration was observed with ferric chloride.

Anal. Calcd. for $C_{28}H_{42}O_7$: C, 68.54; H, 8.63. Found: C, 68.67; H, 8.67.

b. **Chemical Method.**—The crystalline mixture (10 g.) was dissolved in ether (1.5 l.) a small quantity of methanol being added to facilitate dissolution. This solution was shaken with cold 4% aqueous potassium hydroxide solution (4 times 250 ml.) and the combined aqueous layers shaken twice with fresh ether. The combined ether fractions were then washed with water, dried and evaporated to dryness yielding crude **elatericin A** (5.2 g.). Repeated recrystallizations from ethyl acetate–benzene, gave m.p. 149–150° dec. The cold aqueous layers were acidified with cold dilute hydrochloric acid and extracted with ether which after being washed and dried was distilled. Crude **elatericin B** (3.3 g.) was obtained; recrystallizations from the same solvents mixture gave needles m.p. 148–149° dec.

The two compounds separated by both methods were found to be identical in all respects: mixed melting point, same rotation and fingerprint in the infrared.

Elatericin B Diacetate.—Elatericin B (100 mg.) was acetylated overnight at room temperature in dry pyridine (4 ml.) and acetic anhydride (4 ml.). The mixture was decomposed with water and the solid filtered and washed with water. Recrystallization several times from chloroform–petroleum ether produced needles (50 mg.), m.p. 249–250° (red melt), $[\alpha]_D$ −78° in chf. (*c* 0.7); slow formation of color with ferric chloride; ν_{max} 3400, 1730 (ester), 1688, 1628 and 1083 cm.$^{-1}$.

Anal. Calcd. for $C_{32}H_{46}O_9$: C, 66.87; H, 8.07; CH_3CO, 14.9. Found: C, 67.38; H, 7.96; CH_3CO, 14.5.

Elatericin B Triacetate.—Elatericin B (700 mg.) was heated to reflux in acetic anhydride (35 ml.) with freshly fused sodium acetate (1.7 g.) for five hours. The mixture was then decomposed with ice and extracted with ether. The organic layers were collected, shaken with 5% aqueous sodium hydroxide to remove any unreacted material, washed with water, dried and evaporated. The residue (600 mg.) crystallized from dilute methanol as a microcrystalline powder, m.p. 140–142° (hot-stage), $[\alpha]_D$ +48° in chf. (*c* 1.10); ν_{max} 3510 (tertiary OH), 1750 (shoulder for enol acetate), 1723 (s, ester) and 1688 cm.$^{-1}$; no coloration with ferric chloride.

Anal. Calcd. for $C_{34}H_{48}O_{10}$: C, 66.21; H, 7.85; CH_3CO, 20.9. Found: C, 65.97; H, 7.44; CH_3CO, 20.7.

Elatericin B Methyl Ether.—Elatericin B (1 g.), dissolved in acetone (40 ml.), was heated to reflux under nitrogen with dried potassium carbonate (3 g.) and methyl iodide (5 g.) for 72 hours. During this time methyl iodide (5 g. each) was added twice at 24-hour intervals. By this time the mixture gave no coloration with ferric chloride. The solution was filtered, the salt washed with acetone and the filtrates evaporated. The residue was taken with ethyl acetate, shaken with dilute alkali, washed with water, and evaporated after drying over sodium sulfate; leaflets from acetone–petroleum ether, m.p. 217–218°, $[\alpha]_D$ −62° in chf. (*c* 1.70); λ_{max} 231 mμ (ϵ 12,500); new band at ν_{max} 1245 cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.02; H, 8.79; CH_3O, 6.15. Found: C, 68.72; H, 8.77; CH_3O, 5.87.

Diacetate.—The above compound (100 mg.) was acetylated overnight at room temperature in dry pyridine (8 ml.) and acetic anhydride (8 ml.); microscopic needles from chloroform–petroleum ether, m.p. 251–252°.

Anal. Calcd. for $C_{33}H_{48}O_9$: C, 67.32; H, 8.22; CH_3CO, 14.60; CH_3O, 5.26. Found: C, 67.27; H, 8.14; CH_3CO, 14.65; CH_3O, 5.46.

Quinoxalin of Elatericin B.—An alcoholic solution of elatericin B (300 mg.) and *o*-phenylenediamine (300 mg.) was heated under reflux for 24 hours. The colored solution was evaporated and the amorphous orange residue chromatographed through alumina (15 g.) with methanol–ether (1:4). The main fractions could not be induced to crystallize; λ_{max} 239 mμ (ϵ 35,400) and 320 mμ (ϵ 9,900).

Dihydroelatericin B.—Elatericin B (320 mg.) in ethanol solution (20 ml.) was hydrogenated over palladium-on-charcoal (5%, 100 mg.). After seven minutes the amount of hydrogen required for one mole (14.7 ml.) was absorbed and the reaction discontinued. The catalyst was filtered and the solvent evaporated under reduced pressure. The residue crystallized as microcrystals by freezing in a solution of benzene–petroleum ether. Recrystallization gave a product, m.p. 140–143° dec. (shrinking at 120°), $[\alpha]_D$ −22° chf. (*c* 0.9), coloration with ferric chloride; ν_{max} 3450, 1691 (CO), 1662, 1413 and 1088 cm.$^{-1}$; λ_{max} 270 mμ (ϵ 4,600); with alkali: λ_{max} 310 mμ (ϵ 4,100).

Anal. Calcd. for $C_{28}H_{44}O_7$: C, 68.26; H, 9.00. Found: C, 68.52; H, 8.90.

2,4-Dinitrophenylhydrazone, orange crystals from butanol, m.p. 186–188° dec.

Anal. Calcd. for $C_{46}H_{56}O_{16}N_{12}$: C, 53.48; H, 5.42; N, 16.27. Found: C, 53.29; H, 5.49; N, 16.26.

Tetrahydroelatericin B.—The reduction was run as in the previous experiment, same quantities being used. Hydrogenation ceased after 1.7 moles of hydrogen was absorbed. The residue was crystallized twice by freezing in a mixture of xylene–petroleum ether; microcrystals, m.p. 113–116°, $[\alpha]_D$ +32° in chf. (*c* 1.5); no coloration with ferric chloride; ν_{max} 3450 (OH strong) 1710, 1694, 1435, 1388, 1377 and 1090 cm.$^{-1}$.

Anal. Calcd. for $C_{28}H_{46}O_7$: C, 67.98; H, 9.37. Found: C, 67.72; H, 9.08.

The product also crystallized from dilute methanol in prismatic needles, m.p. 174–176° (hot-stage).

Anal. Calcd. for $C_{28}H_{46}O_7 \cdot 0.5H_2O$: C, 66.85; H, 9.42. Found: C, 66.61; H, 9.31.

Hexahydroelatericin B.—An alcoholic solution of elatericin B (1.5 g.) was hydrogenated over Raney nickel catalyst at 65 p.s.i. during 72 hours. Filtration of the catalyst and evaporation of the solvent left a solid which was crystallized from dilute methanol; colorless needles, m.p. 156–157°, $[\alpha]_D$ +49° in chf. (*c* 1.55), no coloration with ferric chloride; ν_{max} 3425 (OH strong), 1693, 1608 and 1099 cm.$^{-1}$; λ_{max} 212 mμ (ϵ 10,000) and 284 mμ (ϵ 160).

Anal. Calcd. for $C_{28}H_{48}O_7 \cdot 2H_2O$: C, 63.13; H, 9.84. Found: C, 63.27; H, 9.53.

The following observation which is ascribed to the presence of a furan ring has been made in the ultraviolet: in alcohol solution (0.1 g. per liter) the absorbance measured at 273 mμ was 0.035. By adding one drop of a 10% solution of alkali to each cell the absorbance increased at the following rate: 0.135 immediately after addition, 0.40 five minutes later and 0.77 in twenty minutes.

The tetraacetate was prepared by acetylating hexahydroelatericin B (800 mg.) overnight at room temperature in dry pyridine (8 ml.) and acetic anhydride (8 ml.); the mixture was diluted with water and the product filtered and washed well with water. Chromatography through alumina (25 g.) gave the following fractions: with benzene–ether 1:1 (300 mg.), ether (traces), and chloroform (180 mg.). The first eluate was crystallized from very dilute methanol; microcrystals, m.p. 135–140° dec.

Anal. Calcd. for $C_{36}H_{56}O_{11}$: C, 65.02; H, 8.49. Found: C, 65.30; H, 8.61.

Reduction of Hexahydroelatericin B. A. By Lithium Aluminum Hydride.—A solution of hexahydroelatericin B (150 mg.) in dry ether (50 ml.) was added to a stirred solution of lithium aluminum hydride (1 g.) in ether (60 ml.). The mixture was heated under reflux for 27 hours and hydrolyzed with dilute acid. The aqueous layer was continuously extracted with chloroform for 24 hours. The ether and chloroform extracts were dried, combined and evaporated. The amorphous residue could not be induced to crystallize; 130 mg.; ν_{max}^{Nujol} 3450, 1697, 1645 and 1087 cm.$^{-1}$.

B. **By Modified Wolff–Kishner Procedure.**[15]—In an all-glass apparatus protected from moisture, sodium (300 mg.) was added to freshly distilled diethylene glycol (15 ml.) and heated to 180° (measured in the liquid). *Anhydrous* hydrazine was distilled until the mixture refluxed at 180°. After cooling, hexahydroelatericin B (500 mg.) was added and the mixture heated overnight under reflux. Some hydrazine was then distilled until the temperature rose to 210° and heating was continued during 24 hours. The mixture

was poured into water and extracted with chloroform. On evaporation, a residue (370 mg.) was left which was chromatographed through activated alumina (20 g.). The following elutions were made: chloroform–benzene 3:2 (50 mg.) and 9:1 (30 mg.), pure chloroform (traces) and chloroform with 1% methanol (60 mg.) and with 5% methanol (100 mg.). All these fractions were amorphous and could not be crystallized. Infrared spectra showed that only in the first fraction the carbonyl absorption had completely disappeared. In all others, weak absorptions at 1697 cm.$^{-1}$ were present and they were probably mixtures.

Elatericinic Acid.—Elatericin B (5 g.) in ethanol (125 ml.) was added to an 8% solution (125 ml.) of sodium hydroxide and heated to reflux under nitrogen for seven hours. Part of the ethanol was evaporated under reduced pressure and diluted with water (50 ml.). The mixture was then acidified with hydrochloric acid and extracted several times with ether. The elatericinic acid formed was extracted with a solution of sodium bicarbonate 5% and after acidification of the solution extracted with ether. Evaporation of the solvent left a residue (2.5 g.) which crystallized from toluene–petroleum ether by freezing, m.p. 140–143° dec. (sinters at 120°).

Anal. Calcd. for $C_{28}H_{44}O_8$: C, 66.11; H, 8.72; mol. wt., 508.6. Found: C, 66.45; H, 8.51, equiv. wt., 505, determined by titration in dry pyridine with tetrabutylammonium hydroxide in benzene–methanol (9:1) using thymol blue as indicator.[21]

(21) R. H. Cundiff and P. C. Markunas, *Anal. Chem.*, **28**, 792 (1956).

The methyl ester of elatericinic acid was prepared with a solution of diazomethane in ether. When the gas evolution subsided, the solvent was evaporated. The residue was amorphous and did not crystallize.

Reduction of Methyl Elatericinate to Elatericinol.—A solution of methyl elatericinate (1.8 g.) in dry ether (100 ml.) was added slowly to a stirred solution of lithium aluminum hydride (3.5 g.) in ether (100 ml.). The mixture was heated for 60 hours and hydrolyzed with dilute acid. The ether layer was washed with water, dried and evaporated leaving a residue (750 mg.). The aqueous layer and the washings were combined and continuously extracted with chloroform for 24 hours yielding on evaporation of the solvent an amorphous substance (450 mg.); microcrystals from xylene, m.p. 127–130° (hot-stage). Both substances were found to be identical by their infrared spectra; ν_{max} 3450 cm.$^{-1}$ (s).

Periodic Acid Oxidation of Elatericinol–Norelatericinone.—Periodic acid (200 mg.) in 3 ml. of water was added to a solution of elatericinol (80 mg.) in ethanol (5 ml.) and left overnight at room temperature. The mixture was then diluted with water and extracted with chloroform. The chloroform layer was washed and distilled, leaving an amorphous substance (56 mg.); ν_{max} 1733 (cyclopentanone) and 1698 cm.$^{-1}$.

Cyclohexanedione was prepared from cyclohexanone and selenious acid in dioxane water according to reference 22: b.p. 78–80° (18 mm.), m.p. 38°, yield 55%.

(22) *Org. Syntheses*, **32**, 35 (1952).

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[CONTRIBUTION FROM THE EMERYVILLE RESEARCH CENTER OF THE SHELL DEVELOPMENT CO.]

The Preparation of Dialkyl Alkylphosphonates by Addition of Dialkyl Phosphites to Olefins

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A peroxide (or light) initiated addition of dialkyl phosphites to olefins is shown to be of considerable utility in the synthesis of dialkyl alkylphosphonates. Some reaction chain lengths have been calculated for three ratios of the reactants—1-octene and dibutyl phosphite. Both olefin and cumene retard the reaction.

The preparation of alkylphosphines by the free radical addition of phosphine to unsaturated compounds has been described in a previous communication.[1] This paper covers an extension of the above study to other compounds having a reactive P–H group, namely, the dialkyl phosphites. The addition of these esters to olefins can also be initiated by actinic radiation or decomposing peroxides and accordingly can likewise be presumed to be free radical in character.[2] The mechanism involves the addition of the phosphite radical to the double bond followed by hydrogen atom abstraction from the phosphite ester.

$$(R'O)_2OPH + R \longrightarrow (R'O)_2OP- + RH$$

$$(R'O)_2OP- + RCH{=}CHR \longrightarrow (R'O)_2OPCHRCHR$$

$$(R'O)_2OPCHRCHR + (R'O)_2OPH \longrightarrow (R'O)_2OPCHRCH_2R + (R'O)_2OP-$$

where R is H or an alkyl radical. A number of examples of this reaction are presented in Table I.

One of the more notable characteristics of the phosphite–olefin systems is the effect of reactant ratios upon reaction rate or, what is its equivalent, chain length. A variation in the reactant ratio not only changes the rate of conversion but also influences the composition of the product. Specifically (*cf.* Tables III and IV), a high concentration of dibutyl phosphite gives a kinetic chain length which is quite large, while with a low phosphite concentration (*cf.* Table II) the kinetic chain length is much less and, in addition, falls off quite rapidly as the reaction proceeds. The composition of the product was determined from the ratio of the consumption of olefin to the consumption of phosphite. In the experiment with a high concentration of olefin, the product contained between two and three olefin units to one phosphite unit, whereas with a low concentration of olefin in the starting material the product contained only slightly more than one olefin unit for every phosphite unit.

The kinetic chain length, which is a measure of peroxide efficiency, is computed from the number of molecules, both phosphite and olefin, reacted per initiating free radical. This latter value is twice the number of molecules of peroxide decomposed, which value in turn is derivable from the rate expression.[3]

(1) A. R. Stiles, F. F. Rust and W. E. Vaughan, THIS JOURNAL, **74**, 3282 (1952).

(2) A. R. Stiles, D. Harman and F. F. Rust, U. S. Patent 2,724,718 (to Shell Development Co.), Nov. 22, 1955.

(3) J. H. Raley, F. F. Rust and W. E. Vaughan, THIS JOURNAL, **70**, 88 (1948).