method described in reference 6. The free base, β-mercaptoethylamine, was prepared by the method of Nathan and Bogert[7]; the hydrochloride used in later experiments was obtained from Evans Chemetics. Unless specified, chemicals were all Eastman Kodak Co. preparations.

Titration Curves.—The titration curves were obtained by dissolving the compound in alcohol, water or a mixture thereof and adjusting the solution to about 30% alcohol. The titrants were 0.1 *N* sodium hydroxide and 0.1 *N* sulfuric acid in water. Measurements were accomplished by using the Beckman model M *p*H meter with glass electrode and silver–silver chloride reference electrode. The curves are plotted in all cases with the neutral or uncharged molecule at the center. In Fig. 2, the *p*H values at 0.5 equivalent gave a rough estimation of the relative *pK*'s of the amines.

(6) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 441.

(7) A. H. Nathan and M. T. Bogert, THIS JOURNAL, **63**, 236 (1941).

ROCHESTER, N. Y.

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

The Constituents of *Ecballium elaterium* L. II. α-Elaterin[1,2]

BY DAVID LAVIE AND SHLOMO SZINAI

RECEIVED AUGUST 19, 1957

α-Elaterin, β-elaterin and elateridin have been found to have anti-tumor activity. The oxygen functions of α-elaterin have been studied, and the formation of ecballic acid, a degradation product of α-elaterin is explained by a benzilic acid type rearrangement. Pyrolysis of α-elaterin yielded a new crystalline degradation product, pyroelaterin.

Ecballium elaterium or squirting cucumber is a trailing plant growing in the Mediterranean region, belonging to the Cucurbitaceae. The fruit resemble small gherkins, but are thickly covered with bristly hairs. As these fruits ripen, the juice surrounding the seeds swells and a pressure is set up within the fruit until the joint between the stalk and the fruit ruptures and the fruit's contents and the seeds are shot out violently by the slightest touch. The juice yields a powerful cathartic drug called "elaterium" which has been used since ancient times for its medicinal properties[3] and was included in the British Pharmacopeia.

The fruit is collected when nearly ripe and the juice expressed after crushing or slicing. From the juice settles a deposit which is collected and dried. From this deposit or "elaterium," Morries and Hennell simultaneously reported the isolation of the crystalline elaterin in 1831.[4] The empirical formula of $C_{28}H_{38}O_7$ was proposed for this compound by Berg.[5] The same author showed that when elaterin is treated for a short time with alcoholic sodium hydroxide, one molecule of acetic acid is split off and an amorphous phenolic substance, elateridin, is formed. Under the prolonged action of boiling sodium hydroxide solution, elaterin is converted into a crystalline product, ecballic acid.[6] From this observation, and from the fact that elaterin was soluble in alcoholic alkaline solution and could be recovered unchanged by immediate acidification, a lactone was assumed to be present in the molecule.[7] Borsche and Diacont[6] also assumed that the prolonged treatment of elaterin with alkali would involve certain changes in the molecule which would not allow relactonization and would lead to the formation of an acid. From the acid hydrolysis of elaterin[5,8] an amorphous product was obtained, anhydro-elateridin. During the purification of crude elaterin, Power and Moore[9] isolated, in small quantities, a second compound, β-elaterin, to which they ascribed the physiological activity of the mixture.

In the course of a systematic screening of plant extracts in a search for substances with tumor necrotizing capacity, Belkin and Fitzgerald,[10a] found that *Ecballium elaterium* possesses marked activity. The neoplasm used in this investigation was Sarcoma 37 implanted intramuscularly into the right hind leg of hybrid CAF_1 mice. When α-elaterin was administered subcutaneously, it did not show any toxicity and had insignificant anti-tumor activity, while β-elaterin was active. When given intraperitoneally both α- and β-elaterin were found to be very active. Both produced tumor damage, the latter being more effective. Elateridin obtained by the hydrolysis of α-elaterin has been found to have also good activity.[10b]

This paper will deal with α-elaterin. The presence of a phenolic group in elaterin is indicated by the brown color which is obtained with ferric chloride, and its solubility in cold alcoholic alkaline solution. Further information regarding the nature of this group was obtained from the study of the ultraviolet spectrum of elaterin. It has a strong peak at 234 mμ (ε 11,700) and a shoulder at 267 mμ (ε 8,350). On addition of sodium hydroxide the shoulder disappears and a new peak is formed at 318 mμ (ε 5,000); this bathochromic shift is reversible with acid. Phenols are known to show shifts of about 50 mμ with alkali, the intensity increasing due to the formation of an enol ion. A decrease in the intensity, of about 40%, which accompanies the bathochromic shift is characteristic

(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) Part I, D. Lavie, *Chemistry & Industry*, 466 (1956).

(3) V. Erspamer, *Riv. ital. essenze profumi piante offic. olii vegetali, saponi*, **28**, 264 (1946); *C. A.*, **41**, 2860 (1947).

(4) Morries, *J. Roy. Institute*, **1**, 352 (1831); Hennell, *Edinb. Med. Surg. J.*, **35**, 339 (1831).

(5) A. Berg, *Bull. soc. chim.*, [3] **35**, 435 (1906).

(6) W. Borsche and K. Diacont, *Ann.*, **528**, 39 (1937).

(7) C. W. Moore, *J. Chem. Soc.*, **97**, 1797 (1910).

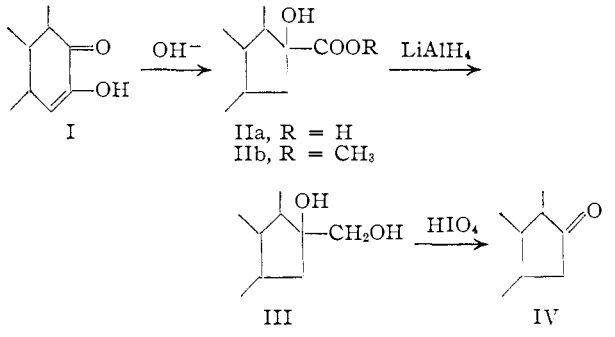
(8) F. v. Hemmelmayr, *Ber.*, **39**, 3652 (1906); A. Berg, *Bull. soc. chim.*, [4] **7**, 385 (1910).

(9) F. B. Power and C. W. Moore, *J. Chem. Soc.*, **95**, 1985 (1909).

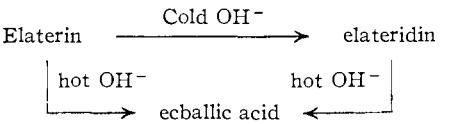
(10) (a) M. Belkin and D. B. Fitzgerald, *J. Nat. Cancer Inst.*, **13**, 139 (1952); (b) the bio-assays were made by M. Belkin and W. Hardy at the National Cancer Institute, Bethesda, Md., and will be published elsewhere.

of the diosphenol chromophore and has been reported by Barton and Eastham.[11] Additional indications for the presence of such a system were obtained from the infrared spectrum. The following bands were found for elaterin: 1727 (ester), 1687 (unsaturated carbonyl), 1660 (diosphenol) and 1628 cm.$^{-1}$; a band at 1413 cm.$^{-1}$ which we believe to be connected with the diosphenol chromophore also was present. The band at 1660 cm.$^{-1}$ and its relation to a diosphenol chromophore will be discussed in the next paper of this series. Elaterin formed a colored quinoxaline derivative with *o*-phenylenediamine, the product showing the characteristic absorptions at 239 and 317 mμ.[12] Thus, the presence of an α-diketone (diosphenol grouping) is further confirmed.

As mentioned earlier, the prolonged treatment of elaterin with alkali leads to the formation of an acid. In this acid, ecballic acid, the phenolic properties of elaterin are lost and the diosphenol system disappears. The formation of this acid may be explained by assuming a benzilic acid type rearrangement of the diosphenol system, an α-hydroxy acid IIa being obtained. A similar observation has been made with buchucamphor.[13] The structure of the acid IIa is supported by the following reactions. Methyl ecballate (IIb) was reduced with lithium aluminum hydride and a glycol III obtained. In this glycol III, all the carbonyl groups originally present in the molecule were reduced, no absorption being found in this region of the infrared. When oxidized with periodic acid, III consumed about one mole of acid, and the amorphous substance IV obtained from the reaction showed a band at 1737 cm.$^{-1}$. This band is characteristic of a cyclopentanone system.[14] Elaterin, then, contains a diosphenol system attached to a six-membered ring.



Saponification of elaterin at room temperature formed elateridin, which was obtained as a microcrystalline substance. Elateridin contained the diosphenol group and its infrared spectrum differed from that of elaterin by the disappearance of the 1727 cm.$^{-1}$ ester band, acetic acid having been split off. Elateridin also rearranged on treatment with hot alkali to ecballic acid. No lactone group could be detected, and it must be assumed that the previous assumptions as to its presence in the molecule were erroneous.



Ecballic acid was polymorphic and could be obtained in two crystalline forms, both forms yielding the same methyl ester with diazomethane.

The empirical formula of elaterin was considered for a long time to be $C_{28}H_{38}O_7$; however, other formulas were advanced at different times.[15] Recently Rivett and Herbstein[16] have determined the molecular weight of elaterin by means of X-ray unit cell and density measurements and have found it to be $C_{32}H_{44}O_8$. The proposed formula for ecballic acid of $C_{26}H_{38}O_7$[6] has now been confirmed by us, by accurate titration in dry pyridine with tetrabutylammonium hydroxide as titrant.[17] Rivett and Herbstein also have found that in addition to the acetic acid split off during the alkali treatment of elaterin, acetoin was obtained. They proposed the over-all reaction with alkali to be

$$C_{32}H_{44}O_8 + 3H_2O \longrightarrow C_2H_4O_2 + C_4H_8O_2 + C_{26}H_{38}O_7$$

The number of oxygen atoms in elaterin is therefore shown to be eight.

Elaterin forms a tris-2,4-dinitrophenylhydrazone, corresponding to three carbonyl groups, two of which are accounted for by the diosphenol (α-diketone). The third carbonyl function is conjugated α-β to a double bond. This is indicated by a peak in the ultraviolet at 234 mμ, and the infrared absorptions at 1687 and 1628 cm.$^{-1}$.

Acetylation of elaterin formed a diacetate. In this compound the phenolic hydroxyl was acetylated; it gave a negative ferric chloride test, and the bands at 1660 and 1413 cm.$^{-1}$ had disappeared. The second acetoxy group was formed by the acetylation of an alcoholic hydroxyl group. A band at 3440 cm.$^{-1}$ in the acetylation product indicated the presence of at least one additional hydroxyl group, presumably tertiary. Methylation of elaterin with methyl iodide and potassium carbonate formed an enol ether, elaterin methyl ether.[18] In this compound the phenolic properties of elaterin had disappeared.

Elateridin formed an orange bis-2,4-dinitrophenylhydrazone. The formation of a bis derivative could indicate that during the formation of elateridin, changes occurred in the molecule in addition to the splitting of acetic acid.

Acetylation of elateridin formed a diacetate. The hydroxyl group originally present in elaterin as an acetate could not be acetylated by the usual procedures. This observation might be explained by the fact that the acetoxy group of elaterin is bound to a tertiary non-acylatable hydroxyl. Additional proof was obtained by heating elaterin un-

(11) D. H. R. Barton and J. F. Eastham, *J. Chem. Soc.*, 424 (1953).

(12) C. L. Leese and H. N. Rydon, *ibid.*, 303 (1955); F. Bohlmann, *Ber.*, **84**, 860 (1951).

(13) J. L. Simonsen, "The Terpenes," Vol. I, Cambridge University Press, London, 1947, p. 450.

(14) J. F. Grove and H. A. Willis, *J. Chem. Soc.*, 877 (1951).

(15) L. Reichel and K. H. Eisenlohr, *Ann.*, **531**, 287 (1937); see also ref. 5, 6, 7 and 8.

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

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

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

der nitrogen above its melting point; acetic acid was then split off quantitatively.

Out of the glassy melt from the pyrolysis of elaterin, a crystalline material was obtained which was named pyroelaterin. This degradation product was found to contain two additional oxygen atoms. These two atoms were probably introduced by the formation of a peroxide during the crystallization. This assumption was supported by the fact that crystalline pyroelaterin gave a positive iodine test with a potassium iodide solution. No other compound in this series behaved in this manner.

Let us consider now evidence as to the number of C-methyl groups in elaterin and elateridin. Kuhn–Roth oxidation of elaterin gave 12.7% and of elateridin 9.1% of C-methyl. These figures are in agreement for four C-methyl in elaterin and for three in elateridin. The difference between these two compounds is of the acetyl group which is oxidized. Therefore, at least three C-methyl groups must be present in elaterin. These were also indicated by a band at 1380 cm.$^{-1}$ in the infrared spectrum.

Acknowledgment.—The authors wish to thank Mrs. R. Tugenhaft for technical assistance, and Dr. S. Pinchas who has determined the spectra and contributed to their interpretation. We are indebted to Mr. A. Yarden for the titration in anhydrous medium and to Pharmaceutical and Chemical Works "Tamar," Rehovoth, for fruit processing.

Experimental

All melting points reported are uncorrected.

Spectrophotometric Measurements.—Ultraviolet absorption spectra were done on a Beckman model DU quartz spectrophotometer in ethanol solution. 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.

Elaterium.—Fruit of *Ecballium elaterium* L.[19] (100 kg.) was chopped, and the juice strained through a sieve, then the pulp was pressed in a screw press. The dark green juice (40 liters) was collected and left overnight to allow the green precipitate of elaterium to settle. The clear supernatant juice was then carefully decanted and the bottom layer centrifuged for 15 minutes. A thick slimy mass was collected and filtered on a funnel with suction. The elaterium was then dried (hot air) and ground to a powder (190 g.).

α-Elaterin.—Elaterium (50 g.), was extracted in a soxhlet with chloroform for 48 hours. The chloroform solution (containing 24.6 g. of extract) was concentrated to about 50 ml. and an equal volume of petroleum ether was carefully added to avoid precipitation. The crystalline material was filtered and dissolved in a large volume of methanol. The solution was decolorized and concentrated to a small volume; white hexagonal plates, m.p. 232–233° dec., $[\alpha]_D$ −59° in chf. (*c* 0.7); ν_{max} 3450, 1723, 1683, 1660, 1627, 1412, 1370, 1130, 1090 and 990 cm.$^{-1}$; λ_{max} 234 mμ (ε 11,700), 267 mμ (ε 8,350) shoulder; with alkali: λ_{max} 234 mμ (ε 11,700), 318 mμ (ε 5,000). In ethanol it gave a strong coloration with ferric chloride.

Anal. Calcd. for $C_{32}H_{44}O_8$: C, 69.04; H, 7.97; CH_3C, 10.79. Found: C, 68.86; H, 8.10; CH_3C, 12.7.

Tris-2,4-dinitrophenylhydrazone was prepared from elaterin (500 mg.), 2,4-dinitrophenylhydrazine (400 mg.) and hydrochloric acid in boiling ethanol. The precipitate (100 mg.) was chromatographed through alumina (5 g.). Elution with chloroform–benzene gave a red powder (60 mg.) with indistinct m.p.

Anal. Calcd. for $C_{50}H_{56}O_{17}N_{12}$: N, 15.32. Found: N, 15.60.

Quinoxaline Derivative.—Elaterin (100 mg.) in ethanol (50 ml.) was heated to reflux with *o*-phenylenediamine (50 mg.) and a drop of sulfuric acid for three hours. The red fluorescent solution was neutralized and evaporated. The colored residue was dissolved in chloroform, shaken with dilute hydrochloric acid, washed with water and the solvent distilled; ultraviolet spectrum of the colored amorphous residue λ_{max} 239 and 317 mμ.

Elateridin.—To a solution of elaterin (2 g.) in ethanol (300 ml.) cooled to 10°, a normal solution of sodium hydroxide (20 ml.) was added and left overnight at room temperature. The mixture was then acidified with acetic acid, and the solvents distilled *in vacuo*. The product was extracted with ether, and the ether layer was washed with a solution of sodium carbonate and several times with water. The residue (1.8 g.), after evaporation of the ether was triturated with dilute ethanol (excess water). The microcrystalline substance was filtered and recrystallized twice from ethanol–water; microcrystals, m.p. 134–135°, $[\alpha]_D$ +7 in chf. (*c* 1.5), coloration with ferric chloride; ν_{max} 3450, 1688, 1662, 1626, 1413, 1141, 1080 and 985 cm.$^{-1}$; λ_{max} 226 mμ (ε 2,900) shoulder and 270 mμ (ε 9,700); with alkali: λ_{max} 230 (ε 5,400), 317 mμ (ε 5,250).

Anal. Calcd. for $C_{30}H_{42}O_7 \cdot H_2O$: C, 67.73; H, 8.34; CH_3C, 8.45. Found: C, 68.10; H, 8.45; CH_3C, 9.1.

Bis-2,4-dinitrophenylhydrazone: orange crystals from ethanol; m.p. 222° dec.

Anal. Calcd. for $C_{42}H_{50}O_{13}N_8$: N, 12.80. Found: N, 12.81.

Quinoxaline derivative, red-brown powder, λ_{max} 239 and 315 mμ.

Ecballic Acid. A. From Elaterin.[6]—Elaterin (5 g.) was heated to reflux in a 2% solution of sodium hydroxide (300 ml.) for three hours. The solution was filtered and acidified with dilute hydrochloric acid. The precipitate formed was filtered and the filtrate extracted with ether. Evaporation of the ether left a residue (2.4 g.) which was crystallized from methanol–water; yield 0.8 g. of small cubical crystals, m.p. 251–252° dec., $[\alpha]_D$ −58° in acetone (*c* 1.0), no coloration with ferric chloride; ν_{max}^{Nujol} 3440 (alcoholic OH), 3140 and 2500 (acidic OH), 1695 and 1000 cm.$^{-1}$; λ_{max} 226 mμ (ε 300), 285 mμ (ε (100).

Anal. Calcd. for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28; mol. wt., 462.5. Found: C, 67.77; H, 8.52; equiv. wt., 464 determined by titration in dry pyridine with tetrabutylammonium hydroxide in benzene–methanol (9:1) using thymol blue as indicator.[17]

The above precipitate was dissolved in a solution of sodium carbonate. The brown solution was filtered and acidified with hydrochloric acid. The product was extracted with ether, and the ether washed with water and dried over sodium sulfate. Evaporation of the solven left a residue which was dissolved in methanol, water was then added until the mixture became cloudy. The oily material slowly crystallized within a few days. The crystalline mass was collected and triturated with ether and methanol. The crystalline substance was recrystallized from methanol–water; yield 0.7 g., leaflets, m.p. 247–248° dec., $[\alpha]_D$ −59° in acetone (*c* 0.10); showed no depression in the m.p. in a mixture with ecballic acid. On recrystallization from methanol–water and seeding, the cubical crystalline form could be obtained.

B. From Elateridin.—Elateridin (1 g.) was treated like elaterin with a 2% solution of sodium hydroxide (80 ml.) for three hours. The same work-up was followed; small cubical crystals from methanol–water; yield (0.3 g.), m.p. 247–249°, $[\alpha]_D$ −57° in acetone (*c* 0.13). A mixture m.p. with a sample of ecballic acid showed no depression. The infrared spectra of both substances were found to be identical.

Methyl ecballate was obtained by treating a solution of the acid (0.4 g.) in methanol (5 ml.) with an excess of a solution of diazomethane in ether. Evaporation of the solvents left a residue which crystallized from a mixture of ether–petroleum ether; yield 0.3 g., needles m.p. 210–212° dec.

(19) The fruit was collected in the vicinity of Jerusalem by the Department of Botany, Hebrew University, Jerusalem. We wish to express our gratitude for their kind coöperation.

Anal. Calcd. for $C_{27}H_{40}O_7$: 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 $C_{33}H_{46}O_8 \cdot H_2O$: 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]

BY DAVID LAVIE AND DAVID WILLNER

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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 PBT 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).