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# The Constituents of *Ecballium elaterium* L. VII. A Side Chain of Elatericin A and α-Elaterin[1,2]

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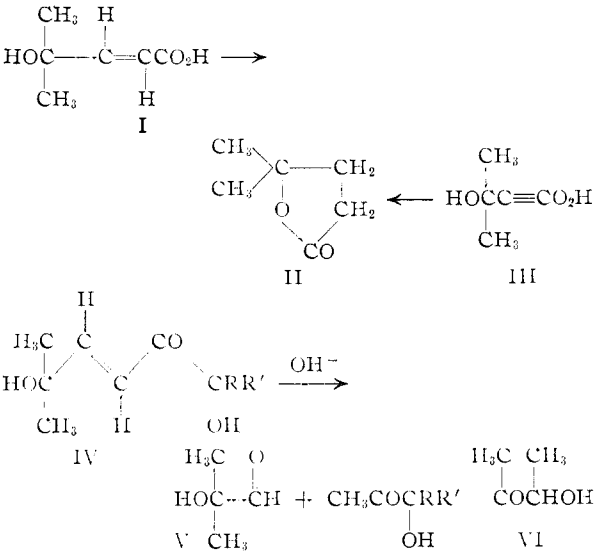
During degradation studies on elatericin A and α-elaterin, *trans*-4-hydroxy-4-methylpent-2-enoic acid has been obtained, thereby elucidating a side chain in these compounds. Seven carbon atoms and three oxygen atoms have been identified in this side chain.

In previous papers[2,3] of this series the functions present in elatericin A and α-elaterin were described. During this investigation elatericin A was oxidized with periodic acid, and two moles of this reagent was consumed.[4] The aqueous layer which remained after the filtration of the amorphous oxidation product, containing the bulk of the molecule, was continuously extracted with ether. By treating the ether residue with chloroform, a crystalline water-soluble acid was obtained. This compound, m.p. 102–103°, which analyzed for $C_6H_{10}O_3$, was identified as *trans*-4-hydroxy-4-methylpent-2-enoic acid (I), not described heretofore, on the following grounds. The ultraviolet spectrum of this acid, $\lambda_{max}$ 208 mμ (ε 9,500), pointed out in a clear way that a double bond was α,β-conjugated to the carboxylic acid group.[5] This was also seen in the infrared spectrum, $\nu_{max}$ 1703, 1651 and 980 cm.$^{-1}$, the last frequency indicating the *trans* configuration of the double bond.[6] Three carbon atoms including the carboxylic group were thereby defined. The remaining half of the molecule should also accommodate a hydroxyl group indicated by a broad band in the hydroxyl region. Hydrogenation of the double bond over platinum yielded a distillable oil which showed a band at 1778 cm.$^{-1}$ in the infrared attributed to a five-membered lactone (II) thus locating the hydroxyl group at the γ-position. Of the two possible structures to be considered for the molecule, a straight chain of carbon atoms or a branched chain having a *gem*-dimethyl group on carbon atom four, the optical inactivity of the compound pointed only toward the second structure (I), which was confirmed by synthesis. 4-Hydroxy-4-methylpent-2-ynoic acid (III) was prepared by treating the Grignard reaction product of but-3-methyl-3-hydroxy-1-yne with carbon dioxide.[7] Repeated attempts to obtain I by reducing stereospecifically the pentynoic acid III with sodium in liquid ammonia[8] were unsuccessful; the acid III was therefore hydrogenated over platinum and the product obtained was isocaprolactone (II).[9] Both synthetic and degraded products melted at 8°, and a mixture of the two compounds did not depress the melting point. Moreover, the infrared spectrum of both compounds were superimposable throughout the whole range.



Acid I, obtained by the periodic acid oxidation of elatericin A, was therefore originated from a side chain having structure IV.

Oxidation of α-elaterin with periodic acid, following the same sequence of experiments, yielded also acid I identifying the same side chain (IV) for this compound. It has been found by Rivett and Herbstein[10] that during the treatment of α-elaterin with hot alkali, a reaction already investigated for the preparation of ecballic acid,[11] acetoin was formed. Acetoin was obtained as the 2,4-dinitrophenylhydrazone of biacetyl, following steam distillation of the alkaline reaction mixture. Repeating this experiment with elatericin A resulted also in the formation of acetoin. The reaction product from α-elaterin, namely ecballic acid, as well as the corresponding amorphous acid obtained from elatericin A, contained a methyl ketone indicated by an iodoform test. Rivett and Herbstein[10] suggested the formation of acetoin by a reto-aldol condensation. We are now in a position to locate this hydrolytic splitting to the double bond which is conjugated to the ketone in the side chain (IV). However, from such a splitting 2-hydroxyisobutyraldehyde (V) should

(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) Part VI, D. Lavie and Y. Shvo, THIS JOURNAL, **81**, 3058 (1959).

(3) D. Lavie and S. Szinai, *ibid.*, **80**, 707 (1958).

(4) D. Lavie and Y. Shvo, *Proc. Chem. Soc.*, 220 (1958).

(5) A. T. Nielsen, *J. Org. Chem.*, **22**, 1539 (1957).

(6) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen & Co., Ltd., London, 1956, p. 40.

(7) L. J. Haynes and E. R. H. Jones, *J. Chem. Soc.*, 505, 954 (1946).

(8) R. A. Raphael, "Acetylenic Compounds in Organic Synthesis," Butterworths Scientific Publications, London, 1955, p. 27.

(9) J. Bredt and R. Pittig, *Ann.*, **200**, 260 (1880); W. A. Noyes, THIS JOURNAL, **23**, 392 (1901); E. D. Bergmann and E. Zimkin, *J. Chem. Soc.*, 3455 (1950).

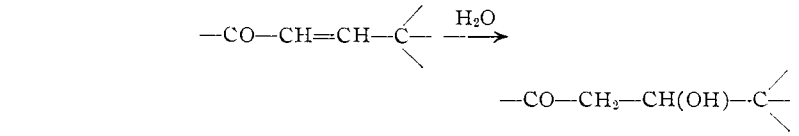
(10) D. E. A. Rivett and F. H. Herbstein, *Chemistry & Industry*, 393 (1957).

(11) W. Borsche and K. Diacont, *Ann.*, **528**, 39 (1937); see also ref. 3.

be expected. An additional step has therefore to be considered toward the formation of acetoin (VI) from 2-hydroxyisobutyraldehyde; this could well be an *acyloin rearrangement*[12a] in alkaline medium; such rearrangements do occur in presence of acids in good yields and have been reported with alkali in the presence of heavy metals oxides.[12b] Since the conditions of our experiments were different, they were repeated with 2-hydroxyisobutyraldehyde heating a synthetic sample in aqueous alkali; acetoin was formed and obtained in the distillate confirming thereby the conditions of the rearrangement.

Confirmation for the structure of side chain IV in α-elaterin were obtained by ozonolysis. The reaction mixture, upon steam distillation, yielded two differently colored 2,4-dinitrophenylhydrazone derivatives. Chromatography on a column packed with acid-washed alumina separated successfully the two components which were identified as the hydrazones of 2-methylacrolein and 2-hydroxyisobutyraldehyde. The formation of 2-methylacrolein can well be explained through a subsequent dehydration of V. Treating the ozonolysis reaction mixture with base or even with sodium bicarbonate resulted in the formation of acetoin.

The elucidation of the side chain of elatericin A and α-elaterin permits now an understanding of the nature and mechanism of reactions described in the previous and preceding papers. The hydrolytic splitting of carbon–carbon bonds has been reviewed by Shemyakin and Shchukina.[13] The pattern of the cleavage of the side chain fits to the proposed mechanism involving the previous addition of a mole of water to the double bond preceding the actual cleavage of the carbon–carbon bond. This addition of water, occurring in presence of alkali, would account for the fast disappearance of the maximum in the ultraviolet at 230 mμ related to the α,β-unsaturated ketone in elatericin A (Fig. 1 of preceding paper[2]). α-Elaterin as well, was found to behave with alkali in the same way, the peak $\lambda_{max}$ 234 mμ disappearing within 3 hours. However, α-elaterin being a naturally occurring ester of acetic acid, formed elateridin[3] upon saponification with cold alkali. It can be stated now that elateridin is not only the saponification product of α-elaterin but also the product of the hydration of the double bond conjugated to the ketone. This explains now the elementary analysis we reported for elateridin,[3] which includes an additional molecule of water ($C_{30}H_{42}O_7 \cdot H_2O$) and the absence of any absorption in the ultraviolet due to an α,β-unsaturated ketone.




Following cleavage of the side chain of elatericin A by periodic acid, the resulting *water-insoluble* moiety of the molecule was investigated. This amorphous product, the bulk of the molecule, possesses one carboxylic acid and at least one aldehyde group giving a positive dimedone test.[14] Two moles of the peracid were consumed during the reaction, oxidizing thereby two oxygenated systems one of which is IV. In view of the formation of a carboxylic acid in the water-insoluble moiety, the second system which is oxidized can be an α-hydroxy-ketone.

Based on oxidations with chromic acid and ozonolysis, Rivett and Enslin recently arrived at similar conclusions regarding the structure of the side chain of cucurbitacin A, C and E (α-elaterin)[15a] thereby indicating the general nature of the side chain for this group of compounds. These authors suggested a tetracyclic triterpenoid structure for these substances[15] since 1,2,8-trimethylphenanthrene was isolated from the dehydrogenation products of cucurbitacin A. Dehydrogenation experiments in our hands on α-elaterin and elatericin A also yielded among other products 1,2,8-trimethylphenanthrene (to be published). We are therefore in a position to support the proposed triterpenoid structure for this whole group of compounds. The identification of a side chain as IV fits to the triterpenoid pattern and places three oxygen atoms in this part of the molecule, leaving four oxygen containing groupings in elatericin A and five in α-elaterin to be located.

**Acknowledgment.**—We thank Dr. P. R. Enslin, National Chemical Research Laboratory, Pretoria, South Africa, for sending us a copy of his paper prior to publication. We are indebted to Dr. S. Pinchas for constructive discussions and interpretation of the infrared spectra. We also wish to express our appreciation to Mrs. R. Tugenhaft for technical assistance and to Mr. E. Meier for the microanalyses.

ADDED MARCH, 1959.—Since this paper was written, Enslin and Norton[13a] oxidized cucurbitacin E (α-elaterin) with periodic acid and reported the isolation of *trans*-4-acetoxy-4-methylpent-2-enoic acid. The acetic ester group[3] is therefore located at the tertiary hydroxyl of the carbon atom bearing the *gem*-dimethyl of side chain IV. This accounts for the low yields of the hydroxy acid I obtained from α-elaterin, owing to the partial hydrolysis of the acetoxy group.

## Experimental

All melting points are uncorrected.

**Spectrophotometric Measurements.**—Ultraviolet absorption spectra were done on a Unicam model S.P. 500 spectrophotometer. Infrared spectra were obtained on a Perkin–Elmer single beam model 12 C spectrometer equipped with a sodium chloride prism.

**Periodic Acid Oxidation. a. Elatericin A.**—To a solution of elatericin A (2.5 g.) in dioxane[16] (50 ml.), periodic acid (4.0 g. of $H_5IO_6$) in water (125 ml.) was added, and the mixture was kept at room temperature for 24 hours. The mixture was then neutralized to *p*H 7 with a dilute solution of sodium bicarbonate and the dioxane distilled under reduced pressure. The remaining solution was acidified with dilute sulfuric acid and the resulting amorphous product was extracted with ether. The ether extract was washed

(12) (a) D. Lavie, Y. Shvo and D. Willner, *Chemistry & Industry*, 1361 (1958); (b) S. Danilow and E. Venus-Danilowa, *Ber.*, **67**, 24 (1934); C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 479.

(13) M. M. Shemyakin and L. A. Shchukina, *Quart. Revs.*, **10**, 261 (1956).

(13a) P. R. Enslin and K. B. Norton, *Chemistry & Industry*, 1959, 162.

(14) N. D. Cheronis, "Micro and Semimicro Methods," Interscience Publ. Inc., New York, N. Y., 1954, p. 466.

(15) (a) D. E. A. Rivett and P. R. Enslin, *Proc. Chem. Soc.*, 307 (1958); (b) P. R. Enslin and D. E. A. Rivett, *J. Chem. Soc.*, 3682 (1956).

twice with water. The combined aqueous fractions and washings were then extracted continuously with ether for 24 hours. This ether extract was collected, dried over sodium sulfate and evaporated to dryness. The residual oil crystallized upon addition of a few ml. of chloroform and cooling. The crystals were filtered and recrystallized a few times from a concentrated solution of the same solvent; yield 0.25 g. of *trans*-**4-hydroxy-4-methylpent-2-enoic acid** (I), prisms, m.p. 102–103°; $\nu_{max}^{CHCl_3}$ wide band for alcoholic and acidic hydroxyls, 1703, 1651 and 980 cm.$^{-1}$; $\lambda_{max}$ 208 m$\mu$ ($\epsilon$ 9,500) (in methanol).

*Anal.* Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75; mol. wt., 130.1. Found: C, 55.37; H, 7.78; mol. wt., 134 (Rast); equiv. wt., 127.

The above ether fraction containing the amorphous water-insoluble acidic fraction of the oxidation was shaken with a saturated solution of sodium bicarbonate. This solution was then acidified and extracted with ether a few times. The ether extract was dried over sodium sulfate and evaporated to dryness leaving an amorphous solid, 1.4 g. This product gave a positive test for aldehydes with methone.[14]

b. **α-Elaterin.**—Powdered α-elaterin (5 g.) was suspended in methanol (240 ml.) and a solution of periodic acid (10 g. of $H_5IO_6$) in water (60 ml.) and sulfuric acid (30 ml. of 5 *N* solution) was added. The mixture was stirred for seven hours and the resulting solution left at room temperature for 48 hours. The mixture was neutralized to *p*H 7 with a 10% solution of sodium carbonate and the solvent removed under reduced pressure. The degradation product was obtained as described in the preceding experiment; yield 0.035 g. of *trans*-**4-hydroxy-4-methylpent-2-enoic acid** (I), prisms, m.p. 102–103°; mixed m.p. with a sample from the previous experiment showed no depression.

**4-Methyl-1,4-pentanolide (Isocaprolactone) (II) from I.**—A solution of *trans*-4-hydroxy-4-methylpent-2-enoic acid (I) (0.18 g.) in ethanol (25 ml.) was hydrogenated over platinum. The amount of hydrogen absorbed was 36 ml. (required for one mole 34 ml.). The catalyst was filtered and the solvent evaporated under reduced pressure. The residue was dissolved in ether and shaken with a cold and saturated solution of potassium carbonate to remove any acid fraction. The ether phase was then dried over sodium sulfate and evaporated to dryness. The residual oil was distilled *in vacuo;* colorless oil, yield 0.06 g., $n^{25}D$ 1.4340, m.p. 7°;[9] $\nu_{max}^{CCl_4}$ 1778 cm.$^{-1}$.

**4-Hydroxy 4-methylpent-2-ynoic Acid (III)**[16].—2-Methyl-3-butyn-2-ol was prepared according to reference 17 by the condensation of acetone and acetylene with sodium amide in liquid ammonia. The product, b.p. 100–103°, $n^{23}D$ 1.4189, was treated with ethylmagnesium bromide then shaken under pressure with solid carbon dioxide. The oil crystallized from chloroform; hygroscopic needles, m.p. 91–92°.[7]

**4-Methyl-1,4-pentanolide (Isocaprolactone) (II) from III.**—4-Hydroxy-4-methylpent-2-ynoic acid (0.5 g.) was hydrogenated over platinum and the reaction product was worked up as described above; colorless oil, $n^{25}D$ 1.4340, m.p. 8°.[9] A mixture with the lactone prepared from I did not depress the melting point. The infrared spectra of both compounds were superimposable throughout the whole range.

**Formation of Acetoin. a. From α-Elaterin.**—Powdered elaterin (1 g.) was heated to reflux in a 1 *N* sodium hydroxide solution (170 ml.) under nitrogen for 6 hours. The yellow solution was steam distilled while nitrogen was passed through the apparatus during the entire operation. The distillate was collected into a solution of 2,4-dinitrophenylhydrazine (0.25 g.) in dilute hydrochloric acid (42 ml. of concentrated hydrochloric acid in 50 ml. of water) to a total volume of 250 ml. The cloudy distillate was left for 24 hours and the precipitate which formed was filtered. The crude hydrazone (0.3 g.) was recrystallized from nitromethane; scarlet red short needles, m.p. 319–322° dec. A mixture m.p. with a sample of **bis-2,4-dinitrophenylhydrazone of diacetyl** showed no depression. The identification of acetoin by this procedure has been described by Rivett and Herbstein.[10]

*Anal.* Calcd. for $C_{16}H_{14}N_8O_8$: N, 25.13. Found: N, 25.32.

b. **From Elatericin A.**—Elatericin A (0.5 g.) was treated like α-elaterin with a 1 *N* sodium hydroxide solution (80 ml.). The same procedure was followed and **bis-2,4-dinitrophenylhydrazone of diacetyl** was obtained, m.p. and mixed m.p. 319–323° dec.

**Ozonolysis of α-Elaterin.**—Ozonization was carried out by passing twice the calculated amount (two double bonds) of ozone through a solution of α-elaterin (1.112 g.) in glacial acetic acid (15 ml.) at 10–15° during 2.5 hours. The solution was diluted with dry ether (200 ml.), cooled to −20° and while passing a slow stream of nitrogen 10 g. of zinc powder were added in portions with vigorous mechanical stirring. The mixture was then stirred for an additional period of two hours at the same temperature. After standing at room temperature overnight, the ether was carefully evaporated using a Vigreux column and after addition of water the reaction mixture was subjected to steam distillation. The distillate was collected into a solution of 2,4-dinitrophenylhydrazine (0.25 g. in 42 ml. of concentrated hydrochloric acid and 50 ml. of water) to a total volume of 250 ml. A stream of nitrogen was passed through the apparatus during the entire operation. The following day the crude hydrazone (0.17 g.) was filtered; it was found to be a mixture of red and yellow crystals. The mixture was dissolved in ethyl acetate (10 ml.) and petroleum ether (90 ml.) and chromatographed through a column of acid-washed alumina[18] (60 g.). Elution with the same solvent mixture yielded firstly the red component (0.10 g.), scarlet red needles from ethanol and nitromethane (a few drops), m.p. 198–200° dec., identified as **2,4-dinitrophenylhydrazone of 2-methyl acrolein.**[19] No depression in mixture m.p. with an authentic sample; $\lambda_{max}$ 367 m$\mu$ ($\epsilon$ 31,400) (chf.).

*Anal.* Calcd. for $C_{10}H_{10}O_4N_4$: C, 48.00; H, 4.02; N, 22.39. Found: C, 47.72; H, 4.31; N, 22.00.

Elution with a mixture of equal volumes of ethyl acetate-petroleum ether yielded the yellow component (0.05 g.), yellow prisms from methanol, m.p. 185–188°, identified as **2,4-dinitrophenyl hydrazone of 2-hydroxyisobutyraldehyde.**[20] No depression in mixture m.p. with an authentic sample; $\lambda_{max}$ 350 m$\mu$ ($\epsilon$ 17,000) (chf.).

*Anal.* Calcd. for $C_{10}H_{12}O_5N_4$: C, 44.78; H, 4.51; N, 20.89. Found: C, 44.80; H, 4.72; N, 20.70.

**Acyloin Rearrangement of 2-Hydroxyisobutyraldehyde.**—2-Hydroxyisobutyraldehyde (0.20 g.), prepared by water hydrolysis of the corresponding bromide[21] was heated to reflux in a 1 *N* sodium hydroxide solution (150 ml.) for two hours. A stream of nitrogen was passed during this time and throughout the whole procedure in the apparatus. The solution was then steam distilled, and the distillate collected into a solution of 2,4-dinitrophenylhydrazine (0.5 g.) in dilute hydrochloric acid (84 ml. concentrated hydrochloric acid in 100 ml. of water) to a total volume of 300 ml. The cloudy solution was left at room temperature for 48 hours to complete precipitation. The crude hydrazone was filtered and dried; yield 0.08 g., needles from nitrobenzene, m.p. 317° dec. A mixture m.p. with a sample of **bis-2,4-dinitrophenylhydrazone of diacetyl** was not depressed.

*Anal.* Calcd. for $C_{16}H_{14}O_8N_8$: C, 43.05; H, 3.16; N, 25.13. Found: C, 43.25; H, 3.32; N, 25.34.

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(16) It should be noted that when methanol was used as solvent, low yields of both soluble and insoluble acids were encountered, probably due to esterification.

(17) *Cf.* "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 320.

(18) Aluminum oxide, Merck, acid-washed; Merck & Co., Inc., Rahway, N. J.

(19) R. W. Brown and G. Dougherty, *J. Org. Chem.*, **13**, 173 (1948). The decomposition point depends on the rate of heating.

(20) K. M. Campbell, THIS JOURNAL, **59**, 1980 (1937), reports m.p. 142° for an unidentified product.

(21) A. Franke, *Monatsh.*, **21**, 215, 1127 (1900).