hours the dark solution was poured over ice, and the resulting acidic solution was washed with ether, made basic with sodium hydroxide, and heated on a steam-bath overnight. The basic solution was extracted with ether. The ethereal solution was dried over sodium sulfate, and dry hydrogen bromide was passed in. The precipitate was removed, dissolved in dilute sodium hydroxide solution, and stirred overnight with 20 ml. of benzenesulfonyl chloride. The solution was acidified to *p*H 1 and the sulfonamide extracted with ether. The acidic solution was then made basic with sodium hydroxide, and was extracted with ether. The ether solution was treated with excess methyl iodide, and the precipitated 1,4-methyliminocyclohexane methiodide (1.5932 g., 10.7%) had m.p. 279–284°. Crystallization from ethanol–methanol gave granular crystals, m.p. 295–295.5° (lit.[50] m.p. 299–300°).

Anal. Calcd. for $C_8H_{16}NI$: C, 37.96; H, 6.37; N, 5.54. Found: C, 37.87; H, 6.60; N, 5.36.

The nuclear magnetic resonance spectrum of the methiodide (D_2O solvent, methylene chloride standard) had three distinct peaks. The peak at +27 c.p.s. (at 40 megacycles) is assigned to the bridgehead hydrogens, the sharp peak at +60.6 c.p.s. is assigned to the N-methyl hydrogens, and the peak at +100 c.p.s. is assigned to the methylene hydrogens. The relative areas under these peaks were, respectively, 2.0, 6.0 and 8.14. Methyliminocyclohexane methiodide requires 2.0, 6.0 and 8.0.

(50) J. V. Braun and K. Schwarz, *Ann.*, **481**, 56 (1930).

In order to compare the rate of decomposition of methylcyclohexylchloroamine with that of dibutylchloroamine under the same conditions, a 0.327 *M* solution of the chloroamine in 90% sulfuric acid in a quartz flask was irradiated with a mercury arc lamp under a stream of nitrogen. The half-life of the chloroamine was about 570 minutes. Under these conditions a 0.28 *M* solution of dibutylchloroamine in 90% sulfuric acid had a half-life of 10 minutes.

Irradiation of N-Chloroazacycloheptane.—N-Chloroazacycloheptane was prepared from azacycloheptane by the same procedure described for methylcyclohexylchloroamine. Approximately 1.8 ml. of the chloroamine was dissolved in 40 ml. of cold 90% sulfuric acid. Titration of a 1-ml. aliquot showed the solution to be 0.38 *M* in chloroamine. The solution was irradiated as with methylcyclohexylchloroamine. The half-life was about 2140 minutes. The solution was diluted with ice-water, made alkaline with sodium hydroxide, and heated on the steam-bath for 6 hours. The alkaline solution was then stirred with 5 ml. of benzenesulfonyl chloride for four hours, and extracted with ether. Dry hydrogen bromide was passed into the ether solution, and the material which separated as an oily suspension was extracted with water. The aqueous solution was made alkaline and extracted with ether. Methyl iodide was added to the ether solution; the oil which separated out (72.6 mg., 3% corrected for aliquots removed) could not be induced to crystallize.

URBANA, ILL.

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

The Constituents of *Ecballium elaterium* L. XI. Proposed Structures for α-Elaterin and its Degradation Products[1,2]

BY DAVID LAVIE AND DAVID WILLNER

RECEIVED AUGUST 5, 1959

α-Elaterin has been identified as a tetracyclic triterpene. A full structure locating all the oxygenated functions is proposed. Structures are also proposed for ecballic acid and other degradation products. The different changes involving the alkaline treatment of α-elaterin are reviewed.

The oxygen functions of α-elaterin (cucurbitacin E), the crystalline compound readily obtained from the fruit juice of *Ecballium elaterium*, have been previously described.[3,4] More recently the side chain of this compound has been elucidated.[5–7] The present paper deals with the full structure of elaterin which is one member of a group of compounds called cucurbitacins[8] isolated from different species of the Cucurbitaceae. An interrelationship has been found among four members of this group through a common degradation product and interconversion.[9] Elaterin as well as elatericin A and B possess anti-tumor activity[10]; a biological investigation is now undertaken to study the different aspects of their action.[11] In view of these properties the full structure proposed for elaterin in this paper might be of interest in the evaluation of new drugs for cancer chemotherapy.

In previous papers[5,6] we have described the periodate oxidation of elaterin and the isolation of *trans*-4-hydroxy-4-methylpent-2-enoic acid. It was therefore proposed that elaterin should have a side chain similar to elatericin A (cucurbitacin D).[2,12] However the yields of this acid were very low when compared to those obtained during the oxidation of the side chain of elatericin A. Sublimation of the oily product, obtained from the mother liquors of the crystallization of the above acid, yielded a crystalline product which was identified as *trans*-4-acetoxy-4-methylpent-2-enoic acid (full details will be published by P. R. Enslin).[7]

(1) (a) This investigation was supported by a research grant C-2810 (C2) from the National Cancer Institute of the National Institutes of Health, Public Health Service; (b) abstracted in part from the doctoral dissertation submitted to The Hebrew University of Jerusalem by David Willner.

(2) Part X, D. Lavie and Y. Shvo, THIS JOURNAL, **82**, 966 (1960).

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

(4) J. N. T. Gilbert and D. W. Mathieson, *Tetrahedron*, **4**, 302 (1958).

(5) D. Lavie, Y. Shvo and D. Willner, *Chemistry & Industry*, 1361 (1958).

(6) D. Lavie, Y. Shvo and D. Willner, THIS JOURNAL, **81**, 3062 (1959).

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

(8) P. R. Enslin, S. Rehm and D. E. A. Rivett, *J. Sci. Food Agric.*, **8**, 673 (1957), and subsequent papers.

(9) D. Lavie, Y. Shvo and D. Willner; P. R. Enslin, J. M. Hugo and K. B. Norton, *Chemistry & Industry*, 951 (1959).

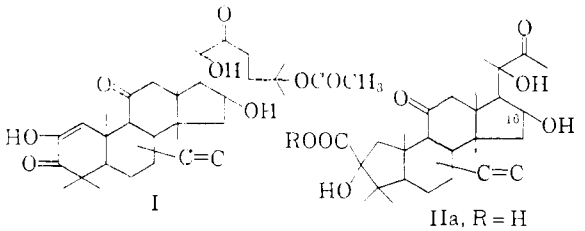
(10) D. Lavie, D. Willner, M. Belkin and W. G. Hardy, presented at the Symposium on the Chemotherapy of Cancer, Tokyo, October, 1957, Abstracts, p. 53; *ACTA, Unio Int. Contra Cancrum*, 15 bis, 177 (1959). Dr. E. Schwenk, The Worcester Foundation for Experimental Biology, Shrewsbury, Mass., kindly informed us that these compounds were found to delay the growth of implanted tumors in the cheek pouch of the hamster.

(11) We thank the National Cancer Institute of the National Institutes of Health, Public Health Service, for an additional research grant C-2810 (C3S) supporting the biological aspects of this investigation. A full report will be published elsewhere.

(12) D. Lavie and Y. Shvo, *Chemistry & Industry*, 429 (1959).

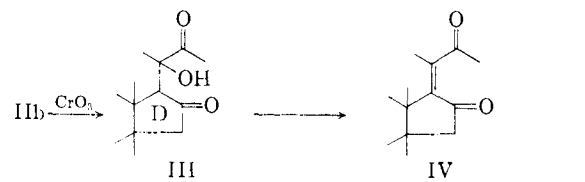
These evidences pointed toward the location of the acetic acid ester group of elaterin in the side chain as shown in I. From earlier acetylation experiments with elaterin and elateridin,[3] the tertiary nature of this ester has already been suggested. Of the eight oxygen atoms of elaterin (I), $C_{32}H_{44}O_8$, four are therefore to be placed in the side chain.[6,13]

In acetic acid solution, using platinum as catalyst, dihydroelaterin was easily obtained. The hydrogenation had to be discontinued when one mole of hydrogen was absorbed. The ultraviolet spectrum of the resulting crystalline product had no maximum at 234 mμ, and it was deduced that the double bond conjugated to the ketone in the side chain of elaterin was reduced during this process.

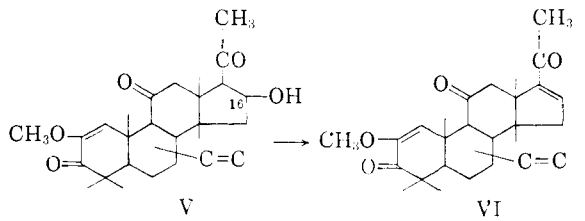


I

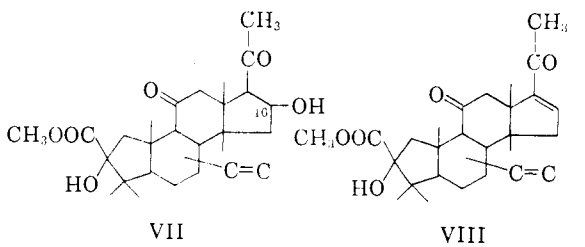
IIa, R = H
b, R = CH_3



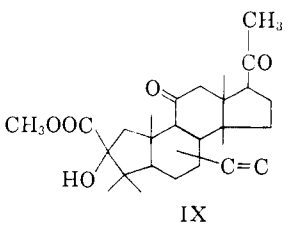
III IV



V VI



VII VIII



IX

In addition to the four oxygen atoms already placed in the side-chain of elaterin (I), two are involved in the diosphenol of the ring system and one acylatable hydroxyl was detected earlier during the acetylation of elaterin (elaterin diacetate).[3,4] The nature of the last oxygen atom in elaterin was now found through a careful study of the infrared spectrum in the carbonyl region. In order to obtain better resolution, a calcium fluoride prism was used, and the 1683 $cm.^{-1}$ frequency previously reported[3] was resolved to its two components. The four carbonyl absorptions, which were now observed were identified as follows: 1724 ester, 1694 hindered ketone, 1688 α,β-unsaturated ketone and 1660 $cm.^{-1}$ for the diosphenol. The 1694 $cm.^{-1}$ frequency is rather low for a saturated ketone, but is in agreement with observations made on ketones in a hindered position.[14] It could not, however, account for a second α,β-unsaturated ketone in the molecule, inasmuch as the ultraviolet spectrum of dihydroelaterin (see above) did not have any absorption in a position required for such a system. This hindered ketone accounts therefore, for the eighth oxygen atom not yet identified in the molecule of elaterin.[13] The unreactive nature of this ketone was already observed during reduction experiments made on this compound; only boiling for a long time with lithium aluminum hydride did reduce it.[3] In agreement with our results, Gilbert and Mathieson[4] observed that ecballic acid formed a bis-2,4-dinitrophenylhydrazone thereby characterizing this group.

Thus, summing up the carbonyl functions of elaterin (I), two are in the side chain forming an α,β-unsaturated ketone and the acetic ester group, the diosphenol system is in a six-membered ring,[3] while in addition there is a sluggishly reactive ketone, probably in a hindered position.

At this stage the study of the dehydrogenation of elaterin (I) was undertaken. For this purpose dihydroelaterin was reduced with lithium aluminum hydride (no carbonyl absorption recorded in the infrared), and the amorphous acetylated product was treated with selenium powder. Among the products of the dehydrogenation, 1,2,8-trimethylphenanthrene was isolated and purified through its trinitrobenzoate adduct. It was identified by the ultraviolet spectrum, and by a mixed melting point with an authentic sample of the adduct, no depression being observed. A second unidentified degradation product forming a 1,3,5-trinitrobenzoate adduct, m.p. 175–178°, was also isolated. The ultraviolet spectrum of this product indicated a tetrasubstituted phenanthrene. Enslin and Rivett[15a] have reported the isolation of similar dehydrogenation products from cucurbitacin A; insofar as the dehydrogenation of tetracyclic triterpenoids has been investigated, they have furnished as main product 1,2,8-trimethylphenanthrene.[15b] In view of these findings, and considering the nature of the side chain, it can be assumed as already suggested by these authors[16] that elaterin has a tetracyclic triterpenoid struc-

(13) D. Lavie and D. Willner, *Proc. Chem. Soc.*, 191 (1959).

(14) A. R. H. Cole and D. W. Thornton, *J. Chem. Soc.*, 1007 (1956); *cf.* also ref. 20a.

(15) (a) P. R. Enslin and D. E. A. Rivett, *J. Chem. Soc.*, 3682 (1956); (b) E. R. H. Jones and T. G. Halsall, "Fortschr. Chem. Org. Naturstoffe," Vol. XII, Springer Verlag, Vienna, 1955, p. 44.

(16) D. E. A. Rivett and P. R. Enslin, *Proc. Chem. Soc.*, 301 (1958).

ture. We may now discuss the location of the remaining groups; namely, one acylatable hydroxyl group, the previously described carbonyl and the diosphenol system.[3,4]

The nature and the location of the hydroxyl group were determined by the oxidation of methyl ecballate (IIb) with chromium trioxide in acetone. It should be noted that in ecballic acid (IIa), which is obtained by the hot alkaline treatment of elaterin (I),[3,4] the side chain has been split hydrolytically at the double bond, leaving a tertiary α-hydroxy-ketone in a shortened chain[6]; simultaneously the diosphenol has been converted to an α-hydroxycarboxylic acid by a benzilic acid-like rearrangement.[3] During the oxidation with chromium trioxide, a new ketone was formed, which absorbed at 1743 cm.$^{-1}$ in the infrared. Evidently, this new band identified the formation of a ketone in a five-membered ring, which could then be placed only in ring D (III), if ring A of the rearranged compound (IIb) is eliminated for such a possibility (see below); indeed it was found to be attached at carbon atom 16 on the following grounds. When the oxidation product (III) was left for 24 hours in an alkaline solution, a new maximum in the ultraviolet was formed at 245 mμ (ϵ 10,500). Such an absorption corresponds very well to an ene-dione system[17] as shown in IV, and in these circumstances was generated by the elimination of a molecule of water from the β-hydroxy-ketone III. It is noteworthy that elimination of the tertiary hydroxyl also happened during chromatography on alumina of the chromium trioxide oxidation product III. It can be deduced therefore, that in elaterin (I), one hydroxyl group is secondary and attached to carbon 16.

In connection with the position of this hydroxyl group, the same conclusion was reached through a different way. Elaterin (I) was oxidized with periodic acid, 2.3 moles being consumed during this reaction.[1b] The two centers of attack were the diosphenol and the tertiary α-hydroxyketone of the side chain (I). Indeed, elaterin diacetate[3,4] still consumed 1 mole of periodic acid, indicating that at least one system involving this oxidation contained a non-acylatable hydroxyl; the diosphenol was made unreactive toward the oxidizing agent by the acetylation of the enolic group. Similarly, the blocking of the diosphenol to periodic acid attack could also be obtained by methylation of the enolic hydroxyl; it was found that elaterin methyl ether[3] consumed only one mole of periodic acid. The same was also true for dihydroelaterin methyl ether ($C_{33}H_{48}O_8$, λ_{max} 264 mμ, 5,000),[18] which was used during this experiment. The product of the periodic acid oxidation of dihydroelaterin methyl ether was chromatographed over alumina, and two crystalline compounds were obtained having the following characteristics: (a) $C_{25}H_{34}O_5$, ν_{max} 3610, 1703 (methyl ketone), 1696 (hindered ketone) and 1644 (enol ether)[19] cm.$^{-1}$; λ_{max} 265 mμ (ϵ 6,000) for the enol ether[18]; and (b) $C_{25}H_{32}O_4$, ν_{max} 1698, 1660 (α,β-unsaturated ketone) 1645 and 1595 (double bond) cm.$^{-1}$, no hydroxyl absorption observed; λ_{max} 244 (ϵ 12,000) and 266 mμ (ϵ 6,500). The two absorptions at 1660 and 1595 cm.$^{-1}$ of the second compound are in agreement with a Δ^{16}-20-ketone system previously described in steroids.[20] From the empirical formulas and the spectroscopic evidences, it was deduced that the former compound still contained the hydroxyl group at position C(16) as shown in formula V, while in the latter, elimination of water had occurred with the formation of an α,β-unsaturated ketone VI. A similar elimination of water involving the hydroxyl at C(16) occurred when methyl ecballate (IIb) was oxidized with periodic acid, the tertiary α-hydroxy-ketone of the side chain being cleaved. The two compounds obtained during this oxidation were methyl bisnor-ecballate (VII): $C_{25}H_{36}O_6$, ν_{max} 1728 (ester), 1708 (methyl ketone) and 1698 cm.$^{-1}$; and methyl-dehydro-bisnor-ecballate (VIII), $C_{25}H_{34}O_5$, ν_{max} 1725, 1698, 1662 (α,β-unsaturated ketone) and 1595 (double bond) cm.$^{-1}$; λ_{max} 239 mμ (ϵ 9,000). Heating VII in benzene solution with p-toluenesulfonic acid resulted in the elimination of the hydroxyl group at C(16) and the formation of compound VIII.[2] Hydrogenation of VIII over palladium resulted in the consumption of one mole of hydrogen and the reduction of the double bond conjugated to the ketone: methyl 16-desoxy-bisnor-ecballate (IX) had no major absorption in the ultraviolet; its infrared spectrum also reflected the saturation of this double bond: ν_{max} 1730, 1702 (methyl ketone) and 1695 cm.$^{-1}$.

The evidences supporting the location of the unreactive carbonyl at C(11) are mainly spectroscopic and based on the hindered nature of this carbonyl. Although position 7 in ring B is also possible, we favor, on the same grounds reported for elatericin A, the C(11) position.[2,13] The location of the double bond in ring B has still to be determined.[9,13]

We now come to the problem of assigning the position of the remaining diosphenol system. For this purpose dihydroelaterin was selected, and the diosphenol was oxidized with hydrogen peroxide in alkaline solution thereby forming a dicarboxylic acid (X). Pyrolysis of this product at 300° resulted in a lively evolution of carbon dioxide. The amorphous residue crystallized after chromatography on alumina, and its infrared spectrum showed a new band at 1740 cm.$^{-1}$ indicative for a ketone in a five-membered ring. According to Blanc's rule, only ring A of a tetracyclic triterpene will account for such a contraction, eliminating therefore rings B and C as possible location for the

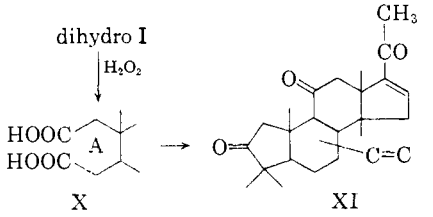
(17) L. Dorfman, *Chem. Revs.*, **53**, 67 (1953); A. Sandoval, J. Romo, G. Rosenkranz, St. Kaufman and C. Djerassi, THIS JOURNAL, **78**, 3820 (1951).

(18) K. R. Hanson, D. B. Jaquiss, J. A. Lamberton, A. Robertson and W. E. Savige, *J. Chem. Soc.*, 4238 (1954). The value found for the enol ether are in good agreement with those reported for quassin and *neo*-quassin which do have such a group. Data concerning the ultraviolet absorption spectra of ethers of enols derived from diosphenols, do not appear to have been recorded previously.

(19) We correlate the absorption at 1644 cm.$^{-1}$ to the enol ether. Such an absorption has also been obtained during methylation experiments on cyclohexanedione and seems not to have been reported earlier.

(20) (a) Cole, "Fortschr. Chem. Org. Naturstoffe," Vol. XIII, Springer Verlag, Vienna, 1956, p. 37. (b) It is noteworthy that the 244 mμ maximum in the ultraviolet of this system is somewhat higher than expected (*cf.* ref. 17), and must be related to the presence of the enol ether of the diosphenol; the same system in VIII has a maximum at 239 mμ.

diosphenol.[21] Formula XI represents the changes occurring during these degradations which were confirmed by the infrared and ultraviolet spectra: ν_{max}^{KBr} 1740, 1695, 1668 (α,β-unsaturated ketone) and 1598 cm.$^{-1}$; λ_{max} 240 mμ (ε 9,000). The diosphenol system is therefore placed in ring A, the enolic hydroxyl occupying position 2 while the ketone the position 3. Such a location accommodates[17,18] the ultraviolet spectrum of elaterin (I), 234 (11,700) and 267 mμ (ε 8,300), and its enol-acetate 231 mμ (ε 19,000) (superimposition of two bands) in elaterin diacetate.[4]



These arguments lead to the expression I for α-elaterin. It is interesting at this point to recapitulate the complicated behavior of α-elaterin toward alkali. During this treatment the side chain is cleaved by a retroaldol condensation forming α-hydroxyisobutyraldehyde which suffers an *acyloin rearrangement*, forming ultimately acetoin as end-product. Simultaneously a *benzilic acid-like rearrangement* takes place in ring A whereby the diosphenol is converted to an α-hydroxycarboxylic acid, the product of these reactions being ecballic acid (IIa).

ADDED JANUARY 1960.—Since this paper was written, Dr. O. R. Gottlieb[21a] has reinvestigated the hydrogenation of elaterin. It was found that in tetrahydroelaterin (in which two moles of hydrogen are absorbed) the diosphenol of ring A was converted to an α-hydroxyketone. The product tetrahydroelaterin methyl ether, previously reported,[13] should be therefore a dihydro derivative and its constants revised. Full details will be published.

Acknowledgment.—The authors thank Mrs. R. Tugenhaft for technical assistance and Mr. E. Meier for the microanalyses. Thanks are also due to Dr. P.R. Enslin, National Chemical Research Laboratory, Pretoria, South Africa. for samples and information prior to publication. Dr. S. Pinchas has determined the infrared spectra with calcium fluoride prism and has contributed to their interpretation. We are indebted to Prof. D. H. R. Barton for a sample of 1,2,8-trimethylphenanthrene.

Experimental

Melting points were taken on a Kofler hot-stage microscope and are corrected. Ultraviolet absorption spectra were done on a Unicam model S.P. 500 spectrophotometer in ethanol solution. Infrared spectra were recorded on a Baird Associates double beam spectrometer equipped with sodium chloride prism. Unless otherwise stated all spectra were determined in chloroform solutions of about 100 mg. per ml. concentration. All optical rotation measurements were carried out in chloroform solution.

Dihydroelaterin.—Elaterin[3,22] (556 mg.) in acetic acid solution (30 ml.) was added to a suspension of reduced platinum oxide (100 mg.) in acetic acid (5 ml.) and hydrogenated at atmospheric pressure. The hydrogenation was discontinued when the calculated amount for one mole of hydrogen (22.5 ml.) was absorbed. The catalyst was filtered and the solvent evaporated under reduced pressure. The residue (540 mg.) crystallized from dilute acetic acid and dilute methanol, as long needles, m.p. 169–172°, $[\alpha]_D$ −45° (*c* 1.6); ν_{max} 1729, 1697, 1657 and 1415 cm.$^{-1}$; the ultraviolet absorption spectrum in neutral solution λ_{max} 269 mμ (ε 6,900); with alkali 0.05 *N* NaOH in 10% aqueous methanolic solution λ_{max} 308 mμ (ε 4,500). In ethanol, coloration was produced with ferric chloride.

Anal. Calcd. for $C_{32}H_{46}O_8 \cdot H_2O$; C, 66.72; H, 8.47. Found: C, 67.10; H, 8.75.

The bis-2,4-dinitrophenylhydrazone was prepared from dihydroelaterin (200 mg.) in methanolic solution as brownish micro-crystals from ethyl acetate–petroleum ether mixture, of undeterminable melting point.

Anal. Calcd. for $C_{44}H_{52}O_{14}N_8$: N, 12.18. Found: N, 11.88.

Dihydroelaterin Diacetate.—Dihydroelaterin (100 mg.) was acetylated in a mixture of acetic anhydride (3 ml.) and dry pyridine (3 ml.) overnight at room temperature. The solution was decomposed with ice-water and the amorphous residue could not be induced to crystallize; λ_{max} 231 mμ (ε 9,500); ν_{max} 1758 (enol-acetate), 1725 (ester) and 1695 cm.$^{-1}$.

Dehydrogenation of Reduced Elaterin. (a) **Reduction of Dihydroelaterin.**—Dihydroelaterin (1 g.) dissolved in tetrahydrofuran (30 ml.) was reduced with lithium aluminum hydride (1.5 g.) in the same solvent (150 ml.) for 48 hours under reflux and stirring. Decomposition with ethyl acetate and a saturated solution of sodium sulfate precipitated most of the salts. The supernatant solution was collected and the precipitate washed with chloroform. The combined solutions were dried over anhydrous sodium sulfate and evaporated leaving a colorless oil (780 mg.). This crude oil was acetylated in acetic anhydride (20 ml.) and dry pyridine (15 ml.) overnight at room temperature. The reaction mixture was decomposed with ice-water and the acetylation product was extracted with chloroform affording an amorphous substance (850 mg.) which could not be induced to crystallize.

(b) **Dehydrogenation with Selenium.**—The above acetylated amorphous substance (3 g.) and selenium powder (8 g.) were intimately mixed and heated at 360° for 50 hours under a stream of nitrogen. The brown reaction mixture was extracted in a soxhlet with ether, and the solution shaken with a 4% sodium hydroxide solution to eliminate any phenolic fraction. The solvent was evaporated affording a brown gum (700 mg.). Distillation *in vacuo* yielded three fractions: fract. 1, b.p. 106–108° (35 mm.), 30 mg.; fract. 2, 134–136° (0.3 mm.), 130 mg.; fract. 3, 160–180° (0.02 mm.), 92 mg. Fraction 1 did not form any adduct with picric acid or 1,3,5-trinitrobenzene. Fraction 2 was chromatographed on alumina (15 g.) to give three petroleum ether eluates: (a) 38 mg. of oil; (b) 37 mg. of oil; both eluates do not form any adduct with 1,3,5-trinitrobenzene; (c) 7.5 mg., semi-crystalline; λ_{max} 254 (45, 000), 261 (51,000), 282 (13,000), 294 (11,000), 306 (13,000), 323 (1,000), 331 (400), 338 (450) and 352 mμ (ε 200) (in isoöctane). The general shape of the curve of this fraction was found similar to 1,2,8-trimethylphenanthrene[15a]; it formed an adduct with 1,3,5-trinitrobenzene, m.p. 185–187°, which was not depressed by authentic, 1,2,8-trimethylphenanthrene 1,3,5-trinitrobenzene adduct. Fraction 3, λ_{max} 262 (42,000), 282 (10,000), 293 (8,000), 306 (8,000) and 338 mμ (ε 400). From the shape of the curve, this fraction appears to be a tetrasubstituted phenanthrene[15a,23]; it formed an adduct with 1,3,5-trinitrobenzene, needles, m.p. 175–178°.

Oxidation of Methyl Ecballate (IIb)[3] to Methyl 16-Oxoecballate (III).—The oxidizing solution was prepared according to Jones[24] (a cold solution of 68 g. of chromic acid in 57 ml. of concentrated sulfuric acid and 100 ml. of water was made up to 250 ml.). Methyl ecballate (300 mg.) was dis-

(21) R. M. Gascoigne and J. J. H. Simes, *Quart. Revs.*, **9**, 328 (1955); L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Corporation, New York, 1949, p. 130.

(21a) In leave of absence from the Instituto de Química Agrícola, Ministério da Agricultura, Rio de Janeiro, Brazil.

(22) The elaterin used in these experiments was also obtained from *Citrullus colocynthis* by enzymatic hydrolysis. Full details will be published.

(23) E. Heilbronner, H. U. Daniker and Pl. A. Plattner, *Helv. Chim. Acta*, **32**, 1723 (1949).

(24) A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.*, 2548 (1953).

solved in redistilled acetone (50 ml.) and the oxidizing solution added dropwise at 0° until a persistent reddish color remained (0.33 ml.). The solution was allowed to warm to room temperature and diluted with ether. The mixture was shaken with water and the organic layer was separated and dried over anhydrous sodium sulfate. Evaporation of the solvent left a residue (300 mg.), crystals from ether, m.p. 185–187°, $[\alpha]_D$ −112° (*c* 0.97), λ_{max} 287–295 mμ (ε 160); ν_{max} 1743 (five-membered ring ketone), 1725, 1704 (carbonyl at C-22) and 1698 cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07. Found: C, 68.13; H, 7.88.

When a 2% sodium hydroxide solution was added to an ethanolic solution of the above product, and the ultraviolet spectrum of the mixture measured 24 hours later: λ_{max} 245 mμ (ε 10,500).

Methyl Dehydro-16-oxo-$\Delta^{17(20)}$-ecballate (IV).—The above product (III) (600 mg.) in benzene was chromatographed over basic alumina (75 g.) (the substance was left in the column for 3 days) and eluted with a mixture of ether and chloroform (1:3). The product which emerged (260 mg.) crystallized from chloroform–petroleum ether, m.p. 248–258° dec., λ_{max} 245 mμ (ε 10,500); ν_{max} 1725, 1700, 1655 and 1617 cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{36}O_6$: C, 71.02; H, 7.95. Found: C, 71.30; H, 7.83.

Dihydroelaterin Methyl Ether.—The etherification of dihydroelaterin (2.5 g.) was made, using the same method described earlier for elaterin.[3] The product (2.5 g.) was microcrystalline from ethanol–water, $[\alpha]_D$ −40° (*c* 0.78); no coloration in ethanol with ferric chloride; λ_{max} 264 mμ (ε 5,000); ν_{max} 3470, 1727, 1710, 1698 and 1645 cm.$^{-1}$.

Anal. Calcd. for $C_{33}H_{48}O_8 \cdot H_2O$: C, 67.09; H, 8.53. Found: C, 66.77; H, 8.93.

Periodic Acid Oxidation of Elaterin and its Derivative.—The titrations were carried out in 80% acetic acid solution according to Siggia.[25] The results are shown in Table I.

TABLE I

Compound	Uptake in HIO_4 in moles in 20 hours
Elaterin	2.30
Elaterin diacetate	0.88
Elaterin methyl ether	1.01
Dihydroelaterin	2.18
Dihydroelaterin methyl ether	1.05
Hexahydroelaterin	2.26
Methyl ecballate	1.15

Periodic Acid Oxidation of Dihydroelaterin Methyl Ether. Compounds V and VI.—Periodic acid (3.8 g.) in 60 ml. of water was added to a solution of dihydroelaterin methyl ether (1.4 g.) in methanol (200 ml.) and left overnight at room temperature. The solution was made neutral with sodium bicarbonate, and most of the solvent was evaporated. The concentrate was diluted with water and acidified with diluted sulfuric acid. The product was extracted with ether (salting out) and the organic layer was dried and evaporated; the oily residue (1.1 g.) was chromatographed over alumina (50 g.) and eluted with the following solvents: (1) benzene, 23 mg.; (2) benzene–ether 1:1, traces; (3) benzene–ether 1:3, 325 mg.; (4) ether, traces; (5) chloroform, traces; (6) chloroform–methanol 5:95, 350 mg. Fraction 3 crystallized from ether, m.p. 193–195°, $[\alpha]_D$ + 14° (*c* 0.77); λ_{max} 244 (12,000) and 266 mμ (ε 6,500); ν_{max} 1698, 1660, 1645 and 1595 cm.$^{-1}$. This substance is **dehydro-hexanor-elaterin methyl ether (VI).**

Anal. Calcd. for $C_{25}H_{32}O_4$: C, 75.72; H, 8.13. Found: C, 75.24; H, 8.22.

Fraction 6 crystallized from methanol–water, m.p. 244–251° dec.; λ_{max} 265 mμ (ε 6,000); ν_{max} 3610, 1703, 1696 and 1648 cm.$^{-1}$. This substance is **hexanor-elaterin methyl ether (V).**

Anal. Calcd. for $C_{25}H_{34}O_5 \cdot {}^1/_2H_2O$: C, 70.89; H, 8.32. Found: C, 70.90; H, 8.36.

(25) S. Siggia, "Quantitative Organic Analysis via Functional Groups," 2nd Edition, John Wiley and Sons, Inc., New York, N. Y., 1954, p. 16.

Periodic Acid Oxidation of Methyl Ecballate. Compounds VII and VIII.—This oxidation was carried out like the previous experiment from methyl ecballate (IIb)[3] (1.2 g.) in methanol (110 ml.) and periodic acid (1.3 g.) in water (35 ml.). The oily residue (1.05 g.) was chromatographed over alumina (50 g.). Elution was made with the following solvents: (1) benzene, 15 mg.; (2) benzene–ether 1:1, traces; (3) ether; (4) chloroform–benzene 1:1, 7 mg.; (5) chloroform–benzene 3:1, 275 mg.; (6) chloroform, 370 mg.

Fraction 5 crystallized from ether, m.p. 199–201°, $[\alpha]_D$ + 32° (*c* 0.8), λ_{max} 239 mμ (ε 9,000); ν_{max} 1725, 1694, 1662 and 1595 cm.$^{-1}$. This substance is **methyl dehydro-bisnor-ecballate (VIII).**

Anal. Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27. Found: C, 72.51; H, 8.20.

Fraction 6 crystallized from benzene, m.p. 225–228°, $[\alpha]_D$ + 60° (*c* 1.2), λ_{max} 283 (100) and 286 mμ (ε 100); ν_{max} 1728, 1708 and 1701 cm.$^{-1}$. This substance is **methyl bisnor-ecballate (VII).**

Compound VII could also be obtained directly, and in a crystalline state, using sodium metaperiodate. This reagent (4.5 g.) in water (100 ml.) was added to methyl ecballate (2.1 g.) in methanol (175 ml.). The mixture was stirred for 24 hours; during this time a precipitate appeared. The solvents were evaporated to a small volume, and water was added to its original volume. The resulting cloudy solution was extracted with chloroform and the lower organic layer was separated, dried over sodium sulfate and evaporated to dryness. The amorphous foam was repeatedly crystallized from ether and from benzene (1.25 g.), m.p. 225–228°; it was found identical with compound VII obtained by the previous method.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.48; H, 8.26.

Dehydration of VII to Form VIII.—To a solution of *p*-toluenesulfonic acid (150 mg.) in benzene (100 ml.) dried by azeotropic distillation, compound VII (400 mg.) was added. The solution was heated under reflux for 2 hours, then cooled and washed with water to neutral reaction. The organic layer was dried over anhydrous sodium sulfate, and evaporated under reduced pressure to dryness. The residue crystallized from ether–petroleum ether yielding 200 mg. of a substance, m.p. 200–202°, $[\alpha]_D$ + 33° (*c* 0.9), identified by mixed m.p. as **compound VIII**; the infrared spectra of this compound and the previously described one were found to be identical throughout the whole range.

Methyl 16-Desoxy-bisnor-ecballate (IX).—Compound VIII (180 mg.) was reduced in ethanol (30 ml.) over palladium-on-charcoal at atmospheric pressure. The hydrogenation stopped when one mole of hydrogen (11 ml.) was consumed. The catalyst was filtered and the solvent was evaporated to dryness. The residue (170 mg.) crystallized from methanol–water, m.p. 163–165°, $[\alpha]_D$ +8° (*c* 0.9); ν_{max} 1730, 1702 (methyl ketone) and 1695 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 72.10; H, 8.75.

Oxidation of Dihydroelaterin to Dicarboxylic Acid X.—To a solution of dihydroelaterin (10 g.) in methanol (300 ml.) cooled to 0° was added a 10% solution of potassium hydroxide (300 ml.). While vigorously stirring the mixture, hydrogen peroxide (33%, 200 ml.) was introduced, keeping the temperature below 5°. Stirring was continued at room temperature overnight. The mixture was then filtered, neutralized with dilute sulfuric acid and excess sodium bicarbonate added. The solution was concentrated *in vacuo* to a reduced volume, water was added to its original volume and filtered from a precipitate. The filtrate was made acidic with dilute sulfuric acid and the resulting cloudy solution was extracted with ether. The ether extract was dried over sodium sulfate and evaporated to leave an amorphous residue (9.2 g.). This product could not be induced to crystallize, it showed acidic properties, and in the infrared two wide bands were observed in the hydroxyl and carbonyl regions; it is therefore the dicarboxylic acid oxidation product X. With diazomethane the dimethyl diester was formed which was also amorphous.

Pyrolysis of Dicarboxylic Acid X to Form XI.—The dicarboxylic acid X (2 g.) was heated to 300° for two hours under a stream of nitrogen and at reduced pressure. Evolution of gas developed immediately. The residue was dissolved in

benzene and chromatographed over alumina (60 g.). The following fractions were collected: (1) benzene, 44 mg.; (2) benzene–ether 3:1, 180 mg.; (3) benzene–ether 1:1, 226 mg.; (4) ether, 86 mg.; (5) chloroform, 340 mg., Fraction 3 only crystallized; repeated crystallizations from ether, m.p. 195–197°, $[\alpha]_D$ + 48° (*c* 0.6), λ_{max} 240 mμ (ε 9,000); ν_{max}^{KBr} 1740 (ketone in five-membered ring), 1695, 1668 (α,β-unsaturated ketone) and 1598 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{30}O_8$: C, 77.93; H, 8.53. Found: C, 77.46; H, 8.79.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Chondroitin Sulfate Modifications. I. Carboxyl-reduced Chondroitin and Chondrosine

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Carboxyl-reduced chondroitin (II) was prepared by exhaustive sodium borohydride reduction of chondroitin methyl ester (I) in borate buffer. Partial acid hydrolysis of II, *N*-acetylation and carbon column fractionation of the hydrolyzate facilitated the isolation and characterization of D-glucose, 2-acetamido-2-deoxy-α-D-galactose monohydrate and the sole disaccharide, 3-*O*-β-D-glucopyranosyl-2-acetamido-2-deoxy-α-D-galactopyranose dihydrate (III). Compound III was readily degraded in alkaline solution to D-glucose and a Morgan–Elson reactive sugar, "anhydro-*N*-acetyl-D-galactosamine." Sodium borohydride treatment of III gave the alditol IV, chromatographically identical to the product derived from chondrosine methyl ester hydrochloride. *N*-Acetylhexosaminols were shown to give positive Morgan–Elson reactions. Formula VIII is proposed as the correct structure for the periodate oxidation product of *N*-acetylchondrosinol (VI).

The acid instability of hexuronic acids has been frequently noted.[2] Owing to the difficulty of attaining complete hydrolysis without destruction of the component monosaccharides, an alternative approach used for studying mucopolysaccharide structure has been to convert the hexuronic acid residues to hexose units by reduction of their methyl esters with sodium borohydride.[3–5] Since no configurational changes were involved, deductions from the hydrolysis of reduced material have been applicable to the original polymer.

We report herein the preparation of 96% carboxyl-reduced polymeric chondroitin (desulfated chondroitin sulfate A) (II) and also carboxyl-reduced chondrosine, the sole disaccharide from the acid hydrolysis of II, isolated as the crystalline *N*-acetyl dihydrate derivative III, m.p. 155–157°, $[\alpha]_D$ +47 → +19° (water). Substance II, $[\alpha]_D$ +11° (dimethyl sulfoxide), was derived, in 71% yield, from chondroitin methyl ester[6] (I) by exhaustive sodium borohydride reduction[7]; the first reduction was 66% efficient, the second increased the reduction to 86% and the third to 96%. These results are in striking similarity to the reduction of the methyl ester of desulfated chondroitin sulfate B[5] with sodium borohydride, where 66 and 85% reductions of the L-iduronic acid to L-idose on the first and second reductions of the polymer were attained.

Partial acid hydrolysis of II, *N*-acetylation and carbon column fractionation of the hydrolyzate, facilitated the isolation of D-glucose, 2-acetamido-2-deoxy-α-D-galactose (*N*-acetyl-D-galactosamine) monohydrate and the sole disaccharide, 3-*O*-β-D-glucopyranosyl-2-acetamido-2-deoxy-α-D-galactose dihydrate (III). D-Glucose was characterized as its crystalline β-pentaacetate and the crystalline 2-acetamido-2-deoxy-α-D-galactose monohydrate was further characterized as the crystalline β-pentaacetate.[8] Carboxyl-reduced chondrosine gave positive ninhydrin and Elson–Morgan[9] reactions. Its crystalline *N*-acetyl derivative III gave a positive Morgan–Elson[10] reaction. On acid hydrolysis of III, D-glucose and 2-amino-2-deoxy-D-galactose (D-galactosamine) were detected by paper chromatography. The infrared spectra of II and III were very similar.

The disaccharide III was degraded readily in dilute alkali solution to D-glucose and a Morgan–Elson[10] reactive sugar ($R_{glucose}$ 1.8), "anhydro-*N*-acetyl-D-galactosamine," different from 2-acetamido-2-deoxy-D-galactose ($R_{glucose}$ 1.2). This is by analogy to the reported[11] alkaline degradation of 3-*O*-β-D-galactopyranosyl-2-acetamido-2-deoxy-D-glucose to D-galactose and "anhydro-*N*-acetyl-D-glucosamine" ($R_{glucose}$ 1.70), different from 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) ($R_{glucose}$ 1.24). The fact[11] that the 4-*O*-β-D-galactopyranosyl analog did not yield "anhydro-*N*-acetyl-D-glucosamine" and the 6-*O*-β-D-galactopyranosyl compound gave, instead, 6-*O*-β-D-galactopyranosyl- "anhydro-*N*-acetyl-D-glucosamine" with $R_{glucose}$ 1.04, should make alkaline degradation a valuable tool for determining *O*-substitution of *N*-acetylhexosamines. 2-Acetamido-2-deoxy-3-*O*-methyl-D-glucose also provided "anhydro-*N*-acetyl-D-glucosamine," which is of unknown structure. 4-*O*- and 6-*O*-substitution for II can be discounted from the results of the alkaline degradation. Its positive Morgan–Elson[10] test eliminates

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