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THE CONSTITUENTS OF *ECBALLIUM ELATERIUM* L—XX
STRUCTURAL TRANSFORMATIONS IN RINGS A AND B IN THE CUCURBITACINS[1,2]

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Abstract—Structural transformations were investigated involving a shift of the double bond from ring B to A induced during the acid treatment of A(2)-nor-hexanor-elatericin A (III). Two isomers were obtained (IV and V) the structures of which were determined by chemical and spectroscopic studies.

THE present paper deals with structural transformations in the cucurbitacins, a group of triterpenoid bitter principles found in the *Cucurbitaceae*. The bulk of our investigation in connection with the structural elucidation of these substances was done on the A-nor-hexanor derivative (III) of elatericin A (I).[4] This degradation product, which had been previously described, was obtained by the periodic acid oxidation of I, which yielded an aldehydo-carboxylic acid (II) in ring A that was subsequently cyclized by heating in sodium carbonate solution. In order to eliminate completely the hydroxyl group at C-16 (which under the experimental conditions was found to be only partially eliminated) the reaction product was treated with *p*-toluenesulfonic acid in benzene solution. The reaction was allowed to proceed for three hours. Following the usual workup the crude product was run on a chromatoplate and two distinct spots were observed indicating the presence of a mixture containing two elimination products. On chromatography two crystalline substances were isolated (each one now producing a single spot on a chromatoplate). The elemental analyses of the two substances were identical indicating that they were isomers. In the new compound the band in the IR spectrum at 1754 cm^{-1} corresponding to a five-membered ring ketone as present in III had now disappeared. Furthermore, the UV absorption spectrum of this new substance showed a peak at 235 mμ with an intensity of absorption of ε 27,500 whereas that of the known isomer (IV) displayed a peak at 240 mμ with an intensity of ε 10,000. This difference in intensity in the order of 17,500 indicates the presence of a new chromophore in addition to the Δ^{16}-20-ketone already present in both isomers. On the basis of the above evidence we propose structural formula V for the new isomer which was also supported by NMR measurements. The vinylic proton at C—1 in compound V couples its spin with the allylic proton at C—5 and is represented by a narrow doublet at 4·36τ (J = 2 c/s). In IV such a proton

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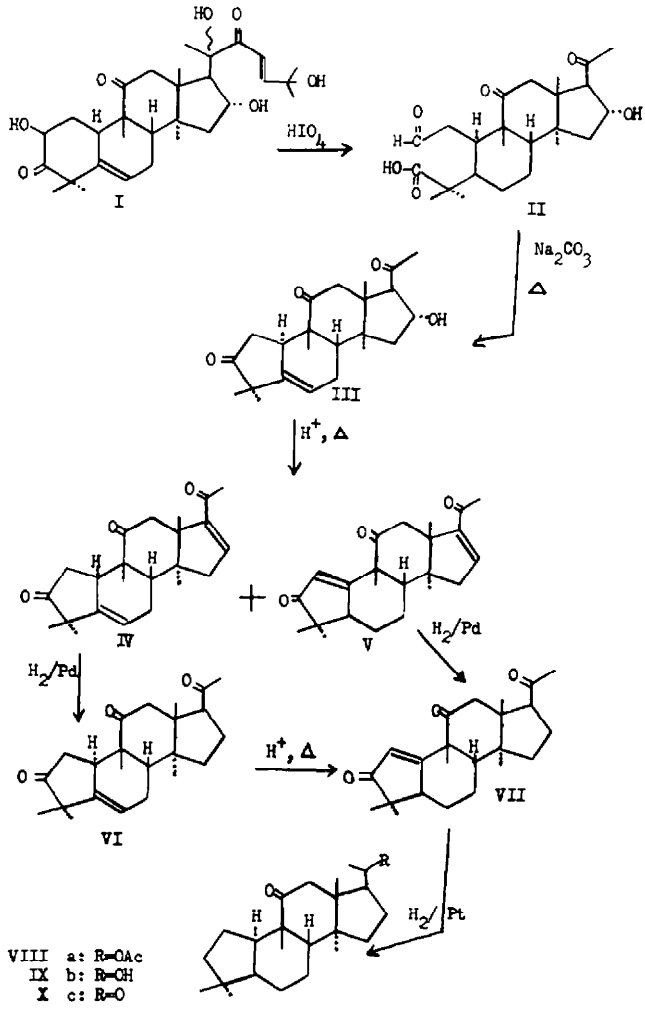
[4] D. Lavie, Y. Shvo, O. R. Gottlieb and E. Glotter, *J. Org. Chem.* 27, 4546 (1962).



does not exist at C—1 and hence only the vinylic proton at C—6 which couples its spin with the neighbouring protons at C—7 and the allylic one at C—10 producing a multiplet (a poorly resolved quartet) centered at 4·27τ.[5] As expected, both isomers displayed triplets at 3·17τ attributed to the C-16 vinylic proton which in each case is split by two neighbouring C-15 equivalent protons. Likewise a good agreement exists between the signals associated with the methyl-ketone groupings (7·69τ) on the side chain. It is noteworthy that in compound IV there is only one proton at C-8 which is not deshielded to any extent due to a vicinal carbonyl group or to a double bond, whereas, there are five such protons in compound V located at carbon atoms 6, 7 and 8. The former appears as a rather wide singlet at 7·79τ accounting for one proton, while the latter are represented by an unresolved and broad peak centering at

[5] A similar unresolved peak 4·68τ had previously been reported.[4] This measurement was taken with a different instrument and no quartet could be observed.

about 8·10τ, the integral of which accounts for five protons. The protons which have not yet been accounted for are those at C-12, while those of the methyl groups will be discussed in a separate paper.[6] A marked difference between the signals related to the C-12 protons of the two isomers was observed, in ketone IV there is a sharp singlet at 6·92τ accounting for two protons, while in ketone V two distinct doublets are found at 6·72 and 7·21τ (J = 17 c/s) corresponding to the α- and β-oriented hydrogens respectively. The peaks of the doublet at higher field were found to be less sharp due to the interaction between the α-hydrogen at C-12 and the neighbouring β-oriented methyl group at C-13. Hence the singlet in IV may be explained by the fact that the C-12 protons in that substance assume equivalent positions with respect to the neighbouring C-11 carbonyl group, a position made possible only if ring C is twisted. We had previously assumed that rings B and C are *cis*-fused. When ring C is in a chair form, as viewed on a model, the methyl group α-oriented at C-14 approaches too closely the α-oriented proton at C-10, whereas a boat form of ring C would bring the two methyl groups at C-13 and C-9 very close to each other, Fig. 1. The

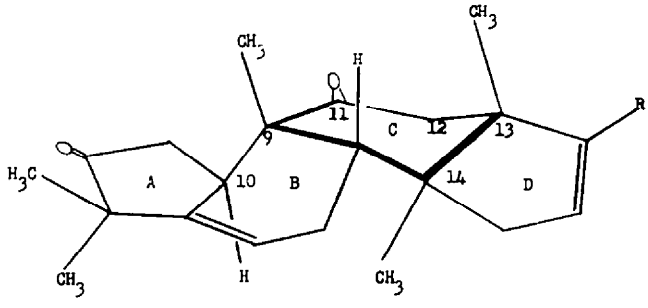


FIG. 1. The shape of Δ^{16}-Desoxy-A (2)-nor-hexanor-elatericin A (IV).

shape which ring C assumes, therefore, can be visualized by a downward movement of the C-11 carbonyl group from a chair form towards the plane formed by the four carbon atoms 8, 9, 12 and 13. A flattening of the ring ensues resulting in an equidistant position of the carbonyl group in regard to the two C-12 protons. Furthermore, such a strained conformation of ring C would also account for the *very strong* positive Cotton effect of this carbonyl group as previously reported.[7] However, compound V does not contain a C-10 hydrogen and hence a chair conformation for ring C can easily be attained which would then result in the expected display of two doublets in the NMR spectrum associated with the two C-12 protons. In order to ascertain the new location of the double bond in V we hydrogenated the triketone IV using Pd–C and thus reducing the 16, 17-double bond. The IR spectrum of the new dihydroderivative (VI) displays the expected band at 1745 cm^{-1} related to the five-membered ring A-nor ketone. The absorption band at 1667 cm^{-1} originally associated with the α,β-unsaturated C-20 ketone[4] had now disappeared and one at 1701 cm^{-1} accommodating the remaining two carbonyl groups was recorded. The UV spectrum indicated only end absorption for the double bond in ring B pointing to the complete reduction

[6] D. Lavie, B. S. Benjaminov and Y. Shvo, Terpenoids IV, *Tetrahedron* **20**, 2585 (1964).
[7] D. Lavie, Y. Shvo, O. R. Gottlieb and E. Glotter, *J. Org. Chem.* **28**, 1790 (1963).

of the double bond in ring D of IV. The NMR spectrum of the reduced product showed an unresolved peak at 4·32τ corresponding to the vinylic proton at C-6, thus implying that the 5,6-double bond had remained intact. The triplet at 3·17τ previously obtained for the C-16 vinylic proton had now disappeared.

Next the dihydro derivative VI was treated with *p*-toluenesulfonic acid in benzene solution in order to bring about a shift of the double bond from ring B to A. This transformation was found to be slow and a total of 75 hr were required to bring it to completion. Following purification of VII by chromatography, the crystalline product displayed a UV absorption maximum at 236 mμ (ε 16,300) indicating the formation of an α,β-unsaturated ketone system, in this case $\Delta^{1(10)}$. The observed intensity of this system conforms very well with the difference between the values of ε 27,500 for compound V and that of compound IV which is ε 10,000 (Δ^{16}-C-20 ketone), this difference being 17,500. Moreover, the IR spectrum of VII showed bands at 1695 and 1590 cm^{-1} for the carbonyl groupings and the double bond respectively. The 1745 cm^{-1} band characterizing the five-membered ring saturated ketone in VI had now disappeared. The NMR spectrum showed unequivocally a narrow doublet at lower field, at 4·42τ (J = 2 c/s), related to the C-1 vinylic proton. Alternatively compound VII was readily obtained in a pure form by the hydrogenation of V using Pd–C. Under these experimental conditions, however, the conjugated double bond in ring A resisted reduction.

In order to bring about the reduction of the double bond in ring A, compound VII, in glacial acetic acid, was hydrogenated using platinum as the catalyst for 52 hr at 70° and high pressure. Under these conditions the carbonyl groups at C-11 and C-20 were expected to undergo reduction as well as possible acetylation. However, the spectroscopic data indicated the formation of VIII. This product, which could not be induced to crystallize, showed a single spot on a chromatoplate. Its IR spectrum showed a peak at 1727 and one at 1258 cm^{-1} both being indicative of the formation of an acetate, and a strong peak at 1689 cm^{-1} which is characteristic of the C-11 carbonyl group in all the cucurbitacins.[4] Moreover, the absence of any UV absorption pointed to the reduction of the double bond in ring A. Hydrolysis of the acetylated compound (VIII) yielded the hydroxy-ketone IX in the IR spectrum of which the acetoxy band did not appear (ν_{max} 3597 cm^{-1} for the hydroxyl group). When this hydroxy ketone was oxidized with chromium trioxide the diketone X was formed. Despite the fact that this was not a crystalline substance it produced a single spot on a chromatoplate in each one of several developing solvent systems. The NMR spectrum of X showed a peak at 7·78τ for the methyl ketone group but no signal at lower field was recorded for a vinylic proton. No UV absorption was observed while the IR spectrum showed a broad band at 1698 cm^{-1} related to the carbonyl groups at C-11 and C-20. No peak of a carbonyl group corresponding to a five-membered ring ketone was found. It can be inferred, therefore, that during the hydrogenation of VII the ketone in ring A was reduced forming an allylic alcohol which, in turn, easily underwent hydrogenolysis and reduction of the double bond yielding thereby the acetoxy ketone VIII.

EXPERIMENTAL

M.ps were taken on a Kofler hot-stage microscope and are corrected. All optical rotations were carried out in chloroform solutions. U.V. absorption spectra were determined in 95% ethanol with a Cary 14 Spectrophotometer. IR spectra were recorded on a Perkin Elmer Infracord model

137 spectrometer equipped with a NaCl prism and were determined in 10% chloroform solutions. NMR spectra were recorded on a Varian A-60 Spectrometer. The spectra were determined in deuterated chloroform solutions of about 5–10% concentration which also contained tetramethyl silane as internal standard. Thin layer chromatography was done on chromatoplates of silica gel G (Merck) and spots were developed with 0·5% $KMnO_4$ in a saturated cupric acetate solution.

Formation of A(2)-*nor-hexanor-elatericin* A (III) *from elatericin* A (I)[8]. To periodic acid (4·8 g) dissolved in water (125 ml), a solution of elatericin A(I) (3 g) in dioxane (80 ml) was added, and the mixture was kept at room temp for 24 hr. Following this, ethylene glycol (5 ml) was added to the mixture to destroy any excess of periodic acid and was allowed to stand for 2 hr. Next, water (80 ml) was added to the mixture and the product was extracted with chloroform (4 × 30 ml). The chloroform layer was washed with a 10% Na_2CO_3 aq (3 × 50 ml) and the combined carbonate washings were heated on a water bath (4 hr) until crystals formed. The mixture was cooled and filtered and the crystals (1·4 g) were washed with cold water and dried in high vacuum; m.p. 210–213°; $[\alpha]_D$ +66° (*c*, 1·65); ν_{max} 1754 and 1698 cm^{-1}; no significant UV absorption; positive iodoform test for a methyl ketone. (Found: C, 74·04; H, 8·67. Calc. for $C_{28}H_{38}O_4$: C, 74·16; H, 8·66%).

Formation of Δ^{16}-*desoxy*-A(2)-*nor-hexanor-elatericin* A (IV) *and* A(2)-*nor-hexanor*-1(10), 16-*cucurbitadiene*-3,11,20-*trione* (V) *from* A(2)-*nor-hexanor-elatericin* A (III)[9]. To a solution of *p*-toluenesulfonic acid (240 mg) in benzene (200 ml) dried by azeotropic distillation, compound III was added. The mixture was heated under reflux for 3 hr using a water collector for the water-benzene azeotrope. The solution was then cooled and washed several times with water until the washings were neutral. The benzene layer was next dried (Na_2SO_4) and evaporated to dryness. The crystalline residue (1·2 g), which showed two spots on a chromatoplate, was dissolved in a small amount of benzene (5 ml) and chromatographed through activated basic alumina (Alcoa F20, 110 g). The following solvents were passed sucessively through the column: benzene (200 ml), ether–benzene (1:3, 2000 ml), ether–benzene (1:1, 400 ml), ether (200 ml). The crystalline fractions of compounds IV and V were obtained during the elution with ether–benzene (1:3). These produced two spots on a chromatoplate. The first 10 fractions which produced the lower spot were combined and recrystallized from ether and the product was found to be IV. Similarly, the last 8 fractions which produced the upper spot on a chromatoplate were combined and recrystallized from ether and were found to be compound V.

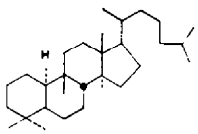
Δ^{16}-*Desoxy*-A(2)-*nor-hexanor-elatericin* A (IV). Needles, m.p. 187–191°; $[\alpha]_D$ +47° (*c*, 1·0); single spot on chromatoplates. ν_{max} 1739, 1698, 1667, 1597 cm^{-1}; λ_{max} 240 mμ (ε 10,000). NMR 4·27τ (unresolved quartet), 3·17τ (triplet), 6·92τ (singlet), 7·69τ (methyl-ketone), 7·79τ (singlet). (Found: C, 77·86; H, 8·20. Calc. for $C_{28}H_{30}O_3$: C, 77·93; H, 8·53%).

A(2)-*Nor-hexanor*-1(10), 16-*cucurbitadiene*-3,11,20-*trione* (V). Prisms, m.p. 209–210°; $[\alpha]_D$ +283° (*c*, 0·9); single spot on chromatoplate. ν_{max}: 1701, 1672, 1592 cm^{-1}; λ_{max} 235 mμ (ε 27,500). NMR 3·17τ (triplet), 4·36τ (J = 2 c/s, doublet), 6·72τ (J = 17 c/s, doublet), 7·21τ (J = 17 c/s, doublet 7·69τ (methyl-ketone), 8·10τ (broad, unresolved peak). (Found: C, 77·94; H, 8·38. Calc. for $C_{28}H_{30}O_3$: C, 77·93; H, 8·53%).

Formation of dihydro-Δ^{16}-*desoxy*-A(2)-*nor-hexanor-elatericin* A (VI) *from* IV. Compound IV (230 mg) dissolved in absolute ethanol (50 ml) was added to a suspension of 10% Pd-C catalyst (50 mg) and was hydrogenated at atm press. The hydrogenation was discontinued after approximately 0·65 mole H_2 had been absorbed. The catalyst was filtered and the alcohol evaporated under red. press. The residue (200 mg) was dissolved in chloroform and recrystallized 3 times from ether, m.p. 172–178°, $[\alpha]_D$ +77° (*c*, 1·0); single spot on a chromatoplate (3:7 ethyl acetate–benzene);

[8] D. Lavie and Y. Shvo, *J. Amer. Chem. Soc.* **82**, 966 (1960), an improved experimental procedure.

[9] The name cucurbitane was suggested for the hydrocarbon skeleton of the cucurbitacins.[7] For the sake of clarity we have kept for the derivatives of elatericin A their original names.



ν_{max} 1745 and 1701 cm^{-1}; UV: weak end absorption. NMR: 4·32τ (C-6, vinylic hydrogen, unresolved peak). (Found: C, 77·50; H, 9·20. Calc. for $C_{33}H_{32}O_3$: C, 77·49; H, 9·05%).

Formation of A(2)-*nor-hexanor*-1(10)-*cucurbitene*-3,11,20-*trione* (VII) *from* V. Compound V (160 mg) dissolved in absolute ethanol (30 ml) was added to a suspension of 10% Pd-C catalyst (40 mg) and hydrogenated at atm. press. After absorbing about 0·46 mole H_2 the reaction was discontinued and the reaction product treated as described above. The residue (146 mg) was dissolved in a small amount of benzene (4 ml) and chromatographed through activated basic alumina (Alcoa F20, 14 g). The fractions obtained upon elution with ether–benzene (1:4) were combined and recrystallized from ether; m.p. 189–192°; $[\alpha]_D$ +304° (*c*, 0·9); single spot on chromatoplate. ν_{max} 1695 and 1590 cm^{-1}; λ_{max} 236 mμ (ε 16,300). NMR: 4·42τ (J = 2 c/s). (C-1) (Found: C, 77·44; H, 9·10. Calc. for $C_{33}H_{32}O_3$: C, 77·49; H, 9·05%).

Acid treatment of 16-*desoxy*-A(2)-*nor-hexanor-elatericin* A (VI). To a solution of *p*-toluenesulfonic acid (15 mg) in glacial acetic acid (20 ml) compound VI (60 mg) was added and the previously described procedure was followed. After 12 hr of heating the product still showed two spots on a chromatoplate and was further treated for 12 more hr. Since two spots were again obtained the crude product was next heated for a total of 75 hr. The product (44 mg) was chromatographed through activated basic alumina (Alcoa F20, 5 g). Elution with benzene (50 ml) was followed by ether–benzene, 1:9 (7 × 25 ml) yielding fractions containing mixtures of VI and VII (30 mg) with ν_{max} 1600, 1698 and 1742 cm^{-1} (the intensity of this band becoming increasingly weaker in the final fractions); λ_{max} 236 mμ (ε 7000, with increasing intensity). The next 4 fractions using the same solvent mixture were combined (10 mg) yielding pure compound VII which was found to be identical in all respects to that obtained from V by its hydrogenation.

A(2)-*Nor-hexanor*-20-*acetoxy-cucurbitan*-11-*one* (VIII). Compound VII (250 mg) dissolved in a mixture of absolute ethanol and glacial acetic acid (50 ml) was hydrogenated at 1300 lbs.in.$^{-2}$ and a temp of about 70° for 52 hr using Pt as the catalyst. Next the catalyst was filtered and the solvents were reduced to a small volume by evaporation *in vacuo*, diluted with water (50 ml) and the product VIII taken up with chloroform, washed with 10% $NaHCO_3$ aq and then with water and finally dried (Na_2SO_4), The chloroform solution was evaporated to dryness yielding a residue (VIII, 224 mg) which could not be crystallized but showing one major spot on a chromatoplate (ethyl acetate–benzene, 3:7); ν_{max} 1727 (shoulder), 1689 and 1258 cm^{-1}; UV: no absorption.

A(2)-*Nor-hexanor*-20-*hydroxy-cucurbitan*-11-*one* (IX). To a sample of VIII (220 mg) dissolved in absolute ethanol (36 ml) a 4% NaOH aq (6 ml) was added and the mixture was allowed to stand for 4 hr. Dilute HCl was added to the reaction mixture to a pH 6–7 and then diluted with water (20 ml) and evaporated to a small volume. The product was extracted with chloroform and the solution washed with water and dried (Na_2SO_4). The chloroform solution was evaporated to dryness to yield a residue (IX, 120 mg); one major spot on chromatoplate (ethyl acetate–benzene, 8:2); ν_{max} 1692 and 3597 cm^{-1}.

A(2)-*Nor-hexanor-cucurbitane*-11,20-*dione* (X). To a stirred ice-cooled solution of IX (77 mg) in purified acetone (25 ml), 0·3 ml CrO_3 aq (68 g of CrO_3 and 57 ml of conc. H_2SO_4 diluted to 250 ml with water) was added dropwise during 30 min. The excess oxidant was destroyed with methanol, water was added and the product extracted with chloroform. The chloroform solution was washed with water and dried (Na_2SO_4). Evaporation of the solvent under red. press. left an oily residue (58 mg). The residue was chromatographed using a column packed with activated basic alumina (Alcoa F20, 5 g). Elution was done with benzene and ether–benzene solvent mixtures. No crystalline fractions were collected. The middle 5 fractions were combined yielding X which showed one spot on chromatoplates using different solvent systems (ethyl acetate–benzene, 3:7; 7% isopropyl alcohol in ethyl acetate and ethyl acetate–benzene, 8:2; ν_{max} 1698 (broad) cm^{-1}, for C-11 and C-20 UV: No significant absorption. NMR: 7·78τ (methyl-ketone), no vinylic proton signal).

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