Tetrahedron Letters No. 18, pp. 615-623, 1961. Pergamon Press Ltd. Printed in Great Britain.

CONSTITUTION OF THE CUCURBITACINS[1,2]

David Lavie,[3a] Youval Shvo, Otto Richard Gottlieb[3b] and
Erwin Glotter
Daniel Sieff Research Institute, the Weizmann Institute of
Science, Rehovoth, Israel

(Received 26 September 1961)

CUCURBITACINS are the various bitter highly toxic principles occurring in the *Cucurbitaceae*. Some have been investigated for their anti-tumor activity.[4]

Based on dehydrogenation experiments and on the nature of the side chain, a regular tetracyclic triterpenoid carbon skeleton has been assigned to them, and structures Ia and Ib have been proposed for elatericin B and elaterin[5] respectively. We now wish to report our experimental results leading to the modification of their structures to IIa and IIb. By previous

---

[1] This investigation was supported by a research grant CY-2810 from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

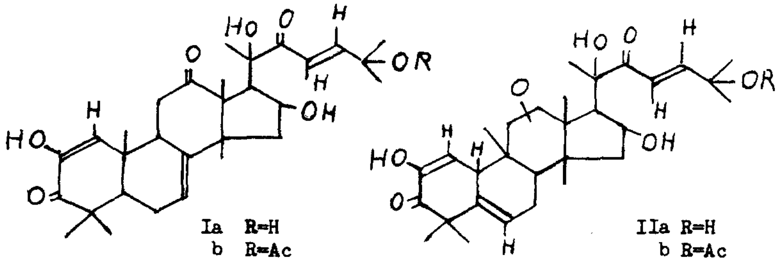
[2] This communication is part XVIII in the series : The Constituents of *Ecballium Elaterium* L., part XVII : D. Lavie, Y. Shvo and O.R. Gottlieb, *Tetrahedron Letters* No. 22, 23 (1960).

[3a] Research fellow at Sloan-Kettering Institute for Cancer Research, New York, 1960-1961; *b* research fellow of the Conselho Nacional de Pesquisas on leave of absence from the Instituto de Química Agrícola, Ministério da Agricultura, Rio de Janeiro, Brazil.

[4] S. Gitter, R. Gallily, B. Shohat and D. Lavie, *Cancer Res.* **21**, 516 (1961).

[5] D. Lavie and Y. Shvo, *Chem. & Ind.* 403 (1960) and ref. 2.

correlation,[6] the structures of elatericin A and cucurbitacin B can be derived.



Ia R=H
b R=Ac

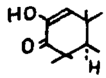
IIa R=H
b R=Ac

Noller, Shoolery et al.[7] have reported nuclear magnetic resonance (NMR) data for diosphenol systems. When a proton was present at the γ carbon atom of such $\alpha\beta$-unsaturated α-hydroxyketone systems, a spin-spin doublet centred at $\delta = 5.68$ was observed. Alternatively, if a hydrogen was not present on the γ' carbon atom, a singlet at $\delta = 6.37$ was recorded. In both cases this signal is attributed to the vinylic hydrogen of the diosphenol system. Since a doublet, centred at $\delta = 5.97$ was found for a compound formed by the air oxidation of dihydro-cucurbitacin B, a hydrogen atom must be present at the γ carbon atom of the newly formed diosphenol system.[7] We were able to confirm these observations using dihydroelaterin (dihydro IIb) which showed a doublet centred at $\delta = 5.97$[10] (J = 2 cps), while a model compound, 4,4-dimethyl cholestane-2,3-dione[8] exhibited a single sharp peak at $\delta = 6.35$. Dihydroelatericin A,[6] in which the ring A double bond is

---

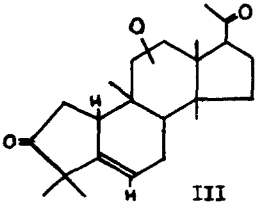
[6] D. Lavie, Y. Shvo and D. Willner; P.R. Enslin, J.M. Hugo and K.B. Norton, Chem. & Ind. 951, (1959).

[7] C.R. Noller, A. Melera and M. Gut; J.N. Shoolery and L.F. Johnson, Tetrahedron Letters No. 15, 15 (1960).

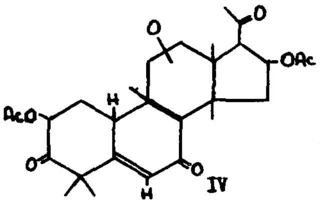
[8] A.P. Sigg and Ch. Tamm, Helv.Chim.Acta 43, 1402 (1960) compound XXVIII  ; G.R. Chaudhry, T.G. Halsall and E.R.H. Jones, J.Chem.Soc. 2725 (1961).

saturated, showed no signals in this region of the spectrum. Since it is impossible to accommodate a diosphenol system with a γ proton in ring A of I, the methyl groups cannot possess the usual tetracyclic triterpene pattern; either a C-4 or the C-10 methyl is missing. A revision of the proposed structure was therefore necessary.

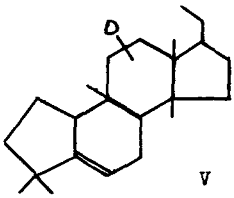
The NMR spectra of 16-desoxy-A(2)-nor-hexanor-elatericin A[5] (III) and cucurbitone D[9a] (IV), as well as other degradation products in which the side chain was removed, all showed five strong signals in the region assigned for methyl groups; an additional strong peak was recorded at δ = 2.08 and is related to a methyl ketone. In no case splittings of these signals could be observed, thus excluding the presence of a secondary methyl ($>CHCH_3$). Consequently it must be concluded that five angular methyls are attached to the carbocyclic skeleton of the cucurbitacins.



III



IV



V

Three out of the five methyl groups should be placed at C-4, C-13 and C-14, since dehydrogenations of the various cucurbitacins[11] yielded

---

[9a] P.R. Enslin, J.M. Hugo, K.B. Norton and D.E.A. Rivett, J.Chem.Soc. 4779 (1960); b A. Melera, M. Gut and C.R. Noller, Tetrahedron Letters No. 14, 13 (1960).

[10] Positions of single peaks and centres of multiplets are expressed in dimentionless "chemical shift" units : $\delta = \frac{\Delta\ (TMS)}{R.f} \times 10^6$ p.p.m.

The term Δ (TMS) is the distance in cps from the tetramethyl silane signal (internal standard) which was taken as zero reference line. The spectra were calibrated by the "side band" technique using an audiofrequency oscillator.

[11] P.R. Enslin and D.E.A. Rivett, J.Chem.Soc. 3682 (1956); D. Lavie and Y. Shvo, J.Amer.Chem.Soc. 82, 966 (1960).

repeatedly 1,2,8-trimethyl phenanthrene. According to extensive previous work, the isolation of this degradation product reveals the presence of methyl groups at C-13, C-14 and C-4 in the original tetracyclic triterpene molecule. In order to satisfy the NMR requirement for the absence of a $>CHCH_3$ grouping, an additional methyl must be secured at C-4, forming a *gem*-dimethyl structure. The presence of such a structure in the molecule was further substantiated by the presence of three absorption bands in the infra-red spectrum of V : 1384, 1377 and 1367 cm^{-1}. The splitting of the hydrogen deformation bands at 1384 and 1377 are in good agreement with the previously recorded observations for the presence of a *gem*-dimethyl group.[12] Compound V was used for this measurement to eliminate the absorption of the methyl ketone in this region of the spectrum; it was prepared by desulphurization of the bis-thioketal of III.

Having ascertained the presence of two methyls in ring A and two between rings C and D, three tertiary positions are left to be considered for the location of the remaining methyl group excluded from C-10, namely positions 5,8 and 9.

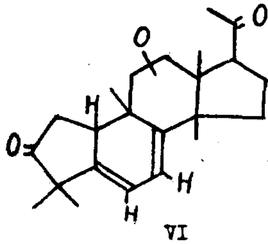
We have repeated the selenium dehydrogenation of elatericin A and isolated, beside the previously reported 1,2,8-trimethyl phenanthrene,[11] a naphthalene derivative possessing an ultra-violet spectrum superimposable on that of 1,2,5-trimethyl naphthalene. The isolation of such a degradation product is indicative of the presence of a methyl group at the B/C rings juncture.[13]

We have previously described[5] the formation of a ring B homoannular diene, λ_{max} 275 $m\mu$ (ϵ = 7800), during the treatment of the bisketal of III

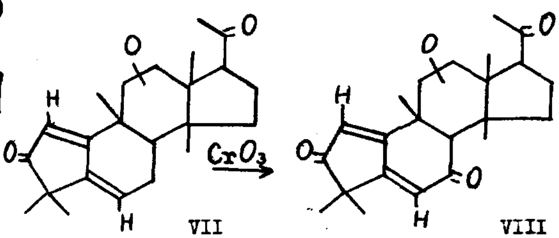
---

[12] L.J. Bellamy, *The Infra-red Spectra of Complex Molecules* p.24 Methuen, London (1959).

[13] D.H.R. Barton *et al.*, *J.Chem.Soc.* 876 (1955); F.G. Fischer and N. Seiler, *Leibigs Ann.* **626**, 185 (1959).



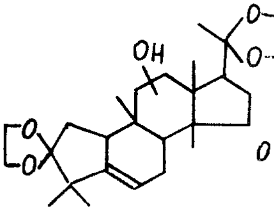
VI

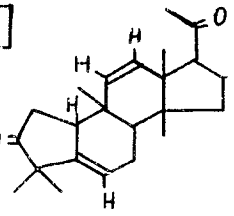


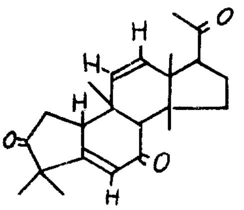
VII VIII

with N-bromosuccinimide. The nature of the diene, having two trisubstituted double bonds was indicated by its infra-red spectrum, a strong new band was recorded at 820 cm^{-1}. This band has been related to the bending vibrations of the two C-H groups at C-6 and C-7. Considering the presence of the methyl groups at C-13 and 14, any other possible homoannular diene in rings B or C should have one <u>cis</u>-disubstituted double bond which should display a band in the 700 cm^{-1} region. However, no absorption was recorded in that region. The NMR spectrum of this diene indicated two doublets centred at δ = 5.42 and 5.62 (J = 4 cps). Only one of these doublets was further split by about 2 cps and, consequently only one allylic proton flanks this diene system. Since it has already been shown that a proton exists at the C-10 position, it follows that C-9 (and C-13) must be occupied by a methyl, and only structure VI (with subsequent hydrolysis of the ketals) can represent this diene. A second product of this reaction, which was obtained in minor quantities, indicated which of the two double bonds of the diene VI corresponds to the original trisubstituted ethylenic linkage in the cucurbitacins. Structure VII, which corresponds to allylic bromination of the C-10 position followed by dehydrobromination, was assigned to this product. The infra-red spectrum of the carbonyl region showed one strong band at 1707 cm^{-1} related to the three carbonyls present in the molecule. This lowering of the five membered ring ketone absorption band, which is present in III (1742 cm $^{-1}$), clearly indicates that it is the five membered ring carbonyl which underwent conjugation. Its ultra-violet spectrum λ_{max} 289 mμ (ϵ = 9000) is consistent with the calculated value. This structure was further supported by oxidation of VII with chromium trioxide in acetic acid yielding a yellow crystalline substance, $C_{23}H_{28}O_4$, m.p. 176-8°, λ_{max} 282 mμ (ϵ = 18,700); ν_{max} 1717, 1709, 1666 and 1569 cm^{-1} to which formula VIII is ascribed. Its NMR spectrum showed two doublets at low field centred at δ = 6.08 and 6.21 (J = 1 cps) and is related to the two vinylic protons which couple their spins, presumably through the conjugated system. Such an allylic oxidation of the nuclear trisubstituted double bond has been encountered in this series of compounds.[9a] The conjugated dienone system in VII clearly locates the inert double bond of the cucurbitacins in ring B between carbon atoms 5 and 6, and also requires a free access for conjugation between rings A and B through carbon atom 10.

In order to adduce complementary evidence for the assignment of a methyl group at C-9, a sequence previously described[5] was reinvestigated. The bisketal of III was reduced with lithium aluminium hydride yielding the hydroxy bisketal (IX), the hydroxyl group being at 11 or 12. Dehydration of IX with phosphorous oxychloride in pyridine at room temperature (with subsequent hydrolysis of the ketals), resulted in the formation of a double bond (Δ^{11}) as shown in formula X. The infra-red spectrum of X showed bands


IX


X


XI

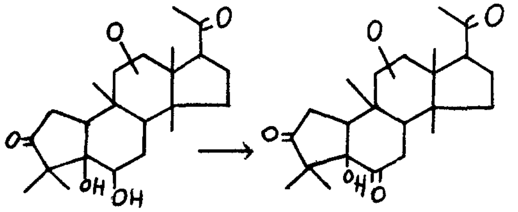
at 1735 and 1708 cm^{-1} related to the five-membered carbonyl and the methyl ketone respectively. In addition a new and strong band appeared at 740 cm^{-1} related to the bending vibration of the newly formed cis double bond. The NMR spectral evidences for X were instructive, since they offered means to study the nature of the two carbons adjacent to the newly formed ethylenic linkage. A symmetrical pattern of lines was observed, forming two doublets centred at δ = 5.28 and 6.00 with equivalent splitting (J = 10 cps). Such a symmetrical pattern of lines was expected for a system of 2 vinylic protons as in 11 and 12 having no vicinal protons for further splitting of the signals by spin-spin coupling. A wide non resolved band was also observed in the vinylic hydrogens region at δ = 5.63. This signal is related to the C-6 proton, and it is unresolved due to spin-spin interaction with the two protons at C-7, and with the allylic proton at C-10 (it is noteworthy that the cucurbitacins, as well as compound III, all exhibit identical unresolved band in the vinylic hydrogen region). In order to indicate the relationship of this unresolved band to the vinylic hydrogen at C-6, the NMR spectrum of the allylic oxidation product of X was analysed. This product (XI), has the following characteristics: ν_{max}^{KBr} 1750 (five-membered ring ketone), 1709 (C-20 carbonyl), 1661 (conjugated carbonyl), 1653 (conjugated double bond), 745 (cis substituted double bond) cm^{-1}; λ_{max} 242 mμ (ϵ = 8720); its NMR spectrum showed again the four line system of the two Δ^{11} protons, while the original unresolved peak appears now as a neat doublet,[14] δ = 5.96 (J = 2 cps). The introduction of the oxygen at C-7 eliminated the spin-spin coupling of the C-7 protons with the vinylic proton, which now couples its spin only with a single allylic hydrogen present at C-10, leading therefore to the expected doublet. The downfield shift of the original signal is

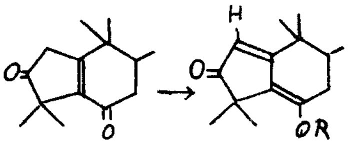
---

[14] L.M. Jackman, Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry p. 85, cf. ref. 9b Pergamon Press, London, New York (1959).

pertinent to the deshielding ability of the carbonyl group. These evidences show once more that the methyl group, previously allocated at C-10 is in fact attached to C-9, and that the nuclear double bond is situated between carbon atoms 5 and 6.

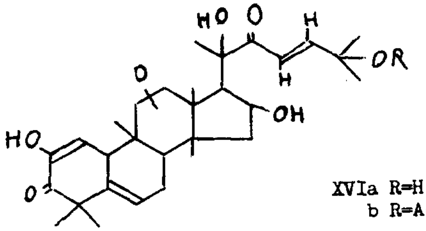
In light of the new observations, a sequence of experiments described earlier[5] and involving the hydroxylation of the nuclear double bond in ring B with OsO_4, was reinvestigated. This hydroxylation reaction led to the secondary-tertiary glycol (XII), which was subsequently oxidized with chromium trioxide in acetone to the tetraketone (XIII). When heated to reflux in benzene with p-toluene sulphonic acid, XIII underwent dehydration. However, instead of an expected neutral product (which can now be formulated as XIV), a crystalline substance with acidic properties was isolated from the reaction mixture: m.p. 200° (decomp.) ν_{max} 3500,1750 (weak), 1700, 1660, 1605 and 1540 cm^{-1}; λ_{max} 335 and 405 $m\mu$ (ϵ = 10,300 and 3,100), $\lambda_{max}^{1\% KOH}$ 405 $m\mu$ (ϵ = 21,700). The system proposed in the previous formulation,[5] which would be consistent with the above experimental data, involved rings B and C. The band observed at 1750 cm^{-1} was thought to be indicative of a non-conjugated five-membered ring ketone in the molecule. However, careful comparisons of the absorption intensities of the different bands indicated that this band is in fact of a much weaker intensity than would be expected for a carbonyl group. This was also true for the intensity of the band at


XII XIII


XIV XVa R=H
b R=Ac

1660 cm^{-1} which is now related to the αβ-unsaturated carbonyl at C-6 (XIV). The spectrum of the reaction product was measured in chloroform, and apparently, the solution must contain a mixture of the two tautomeric forms XIV and XVa. Indeed when this product was acetylated, XVb was obtained in pure form, its infra-red spectrum showed the following major bands: 1765 (enol acetate), 1707 (strong), 1557 cm^{-1}. No absorption band for a saturated five membered ring ketone was observed, as well as no indications were present for an αβ-unsaturated carbonyl in a six-membered ring. The NMR spectrum of XVb showed a single line at $\delta = 5.55$ for the vinylic proton situated at C-1.

In view of these observations we propose to revise the structures of elatericin B and elaterin to IIa and IIb, the hindered carbonyl being situated at either C-11 or C-12. Consequently elatericin A and cucurbitacin B, which have been previously related to these substances[6] have structures XVIa and XVIb.



XVIa R=H
b R=Ac

The relative configurations of several asymmetric centres in the cucurbitacins have been established. These results are not included in this communication, and the formulae as written here are without stereochemical implications.