The residue from the distillation was recrystallized four times from aqueous ethanol to give 430 mg. of the starting quinazolone (m.p. 159–160.5°) and 350 mg. of 3-phenyl-4-(3H)quinazolone, m.p. 142–142.5°, reported[16] 139°.

Control Experiment.—To a solution of 0.90 g. of 1-N,N-dimethylaminopropane[17] in 100 ml. of absolute ethanol, 2.0 g. of W-6 Raney nickel catalyst[14] was added and the resulting mixture was heated under reflux for 90 minutes. Dimethyl-*n*-propylammonium picrate, 480 mg., m.p. 111–112°, reported[18] 108–109°, was isolated by the procedure described above.

Desulfurization of 2-N,N-Dimethylamino-1-propylthiuronium Chloride Hydrochloride.—To a solution of 1.0 g. of 2-N,N-dimethylamino-1-propylthiuronium chloride hydrochloride (m.p. 202–204°) prepared by the method of Renshaw, Dreisbach, Ziff and Green[19] in 100 ml. of absolute ethanol, 2.0 g. of W-6 Raney nickel catalyst[14] was added and the resulting mixture was heated under reflux for 90 minutes. The catalyst powder was removed by filtration and the filtrate was distilled through a short-path still. A saturated solution of picric acid in 95% ethanol was added to the first 50-ml. fraction of the distillate until the solution was neutral to moist litmus. Concentration of this solution gave 270 mg. of dimethylisopropylammonium picrate, m.p. 242–243° dec., reported[15] 240–241° dec.

Acknowledgment.—The authors gratefully acknowledge the generous financial support of the Smith, Kline and French Laboratories, Philadelphia, Penna.

(16) C. Paal and M. Busch, *Ber.*, **22**, 2683 (1889).

(17) See L. Spialter and J. A. Pappalardo, *J. Org. Chem.*, **22**, 840 (1957).

(18) W. Hanhart and C. K. Ingold, *J. Chem. Soc.*, 997 (1927).

(19) This compound was assigned the 1-N,N-dimethylamino-2-propylthiuronium chloride hydrochloride structure by these workers.

LAWRENCE, KAN.

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

The Constituents of *Ecballium elaterium* L. X.[1,2] Proposed Structures for Elatericin A and B

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Elatericin A has been oxidized with bismuth oxide to elatericin B. These naturally occurring compounds have been identified as tetracyclic triterpenes. Full structures are proposed on the basis of various degradation products.

The oxygen functions and the side chain of elatericin A (Ia) (cucurbitacin D) have been previously described,[3–6] and one oxygen atom out of seven was left undetermined. It is the object of the present paper to identify all the functions, and discuss their respective positions in a proposed full structure. In view of the strong tumor necrotizing properties of this group of compounds,[7a,8] the results of this investigation which culminate with the presentation of a full structure are of interest in the chemotherapy of cancer.

Elatericin A belongs to a group of compounds, isolated from different species of the Cucurbitaceae, for which the general name of cucurbitacins has been proposed.[9] Inasmuch as the respective interrelationship among four of these compounds has been proved by interconversion and a common degradation product, the elucidation of the structure of elatericin A provides information regarding most of the structural features of the whole group.[10] Since 1,2,8-trimethylphenanthrene was isolated from the selenium dehydrogenation of elatericin A, the latter substance as well as cucurbitacin A, C and E (α-elaterin) have most probably tetracyclic triterpenoid structures.[11] Furthermore, the identification of the side chain as shown in Ia supports such a structure, and accounts for three oxygenated groups in that chain.[6]

During periodic acid oxidation of elatericin A (Ia) two moles of the reagent are consumed, one mole being used up for the cleavage of the side chain. The hydroxyl group vicinal to the α,β-unsaturated ketone in this chain, has been previously assumed to be secondary.[3] This assumption was made when it was observed that elatericin A diacetate (Ib) seemed to withstand periodate oxidation. However, it was found subsequently that an uptake of the oxidizing agent does occur at a very slow rate.[12] The water-insoluble moiety of the periodic fission, the bulk of the molecule, possessed a methyl ketone, and was a diacetate which analyzed for $C_{28}H_{38}O_7$; it gave a positive iodoform test, and the infrared spectrum of the carbonyl region displayed bands at 1729 (broad) and 1700 cm.$^{-1}$ (overlapping of hindered and methyl ketone); no hydroxyl or aldehyde bands were recorded; the ultraviolet spectrum showed a weak maximum at 288 mμ (ϵ 200). This oxidation product, hexanorelatericin A diacetate, has therefore structure IIb, and the origin of the methyl ketone is the tertiary hydroxyl neighboring a methyl group in the side chain as shown in I.

(1) 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.

(2) Part IX, D. Lavie and D. Willner, *Proc. Chem. Soc.*, 191 (1959).

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

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

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

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

(7) (a) D. Lavie and D. Willner, *ibid.*, **80**, 710 (1958). (b) In this publication a wrong interpretation of the acetylation product of elatericin B has been given. A careful examination of the infrared spectrum of the diacetate taken in KBr pellet disclosed a shoulder at 1757 and a band at 1200 cm.$^{-1}$ for an enol acetate. Further, the ultraviolet absorption maximum at 231 mμ (ϵ 20,500) indicated unequivocally such a system. Of the two acetoxy groups occurring in elatericin B diacetate, one is therefore an enol acetate.

(8) 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).

(9) P. R. Enslin, S. Rehm and D. E. A. Rivett, *J. Sci. Food Agric.*, **8**, 673 (1957), and other papers in this series.

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

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

(12) D. Lavie and Y. Shvo, *Chemistry & Industry*, 429 (1959); this reference is part VIII in this series.

Treating IIb with *p*-toluenesulfonic acid in boiling benzene resulted in a monoacetate ($C_{26}H_{34}O_5$) having now an α,β-unsaturated ketone indicated by the spectroscopic characteristics, λ_{max} 240 mμ (ϵ 10,000) and new bands in the infrared at 1660 and 1590 cm.$^{-1}$. The rather low absorption of the α,β-unsaturated carbonyl in the infrared, and the position of the C=C stretching vibrations are in good agreement with a Δ^{16}-20-ketone reported in steroids[13]; moreover the ultraviolet spectrum also corresponds to such a system (see ref. 16a). Such an elimination was made possible only when the acetoxy group was beta to a ketone[14] as in IIb and did not occur in elatericin A previous to its oxidation. These results require a secondary hydroxyl group at position 16 in ring D of the proposed tetracyclic triterpene, and the new double bond is the product of the elimination of acetic acid resulting in structure IIIb. This compound could also be obtained by the action of dilute hydrochloric acid on IIb and the subsequent reacetylation of the remaining hydroxyl group formed by hydrolysis of the acetate.[12]

We have previously assumed that elatericin A (Ia) possesses an α-hydroxy-ketone in a ring, and discussed its base-induced autoxidation to an α-diketone (diosphenol).[5] This ketol system has been now selectively oxidized with bismuth oxide in acetic acid,[15] and the resulting crystalline compound has been identified as elatericin B (IVa). Elatericin B[7a] (cucurbitacin I), which contains an α-diketone, has been isolated together with elatericin A from the juice of the fruit of *Ecballium elaterium* and other plants.[9] The two compounds, obtained from natural sources, and from the bismuth oxide oxidation, were found to have the same m.p. and mixed m.p., 148–149° dec.; same optical rotation $[\alpha]_D$ − 52°; and their respective infrared spectra were superimposable throughout the whole range. The selective oxidation of elatericin A (Ia) to elatericin B (IVa) with bismuth oxide demonstrates a relationship of an α-hydroxy ketone in elatericin A (Ia) to an enolized α-diketone (diosphenol) in elatericin B (IVa), the remaining part of the molecule being identical.

The location of these two systems, the α-ketol and the diosphenol in elatericin A and B, respectively, had now to be considered. In all known tetracyclic triterpenes, oxygen is found at C(3). From all the oxygenated functions described hitherto, placing the α-ketol and the diosphenol, respectively, in ring A of elatericin A and B appears the most likely possibility. This was supported by an ultraviolet study of dihydro-elatericin B, (Va) and its acetate. Following the uptake of one mole of hydrogen, over palladium, dihydroelatericin B (Va) was formed.[7a] In this compound the α,β-unsaturated ketone of the side chain was reduced to a saturated ketone, coinciding with the disappearance of the maximum at 234 mμ related to the former system. The absorption maximum of the diosphenol chromophore at 270 mμ (ϵ 4,600) was thereby clearly revealed; acetylation of dihydroelatericin B (Va), yielded a diacetate (Vb), ultraviolet λ_{max} 231 mμ (ϵ 7,000), for the enol acetate. The absorptions at 270 and 231 mμ are in close agreement with the respective absorptions of a 2-hydroxy-3-oxo-Δ^1 steroid, and a 2-acetoxy-3-oxo-Δ^1-steroid; such systems in ring B or C would expect to have an absorption maximum at higher wave lengths.[16a,b] Additional proof for the location of the diosphenol in ring A of elatericin B (IVa) was obtained, when it was found that in elaterin the diosphenol is in ring A.[2] The relationship between elatericin A and B to elaterin (cucurbitacin E) has been proved unequivocally through a common degradation product in which ring A was left unaltered.[10]

In order to develop further the structural position of the ketol system in elatericin A (Ia), we must revert to the periodic acid oxidation experiments made with elatericin B diacetate (IVb).[7b] The product of this oxidation was hexanor-elatericin B diacetate (VIb) which analyzed for $C_{28}H_{36}O_7$ and differed from its elatericin A analog IIb by two hydrogen atoms. Since the enol acetate, λ_{max} 231 mμ, ν_{max} 1757 cm.$^{-1}$, could not occupy the position C(3) due to the neighboring *gem*-dimethyl group in C(4), the only alternative position is in C(2) of ring A (VIb). When VIb was hydrogenated over palladium-on-charcoal, one mole of hydrogen was absorbed resulting in the reduction of the enolic double bond, and IIb was formed. This product was identical with hexanor-elatericin A diacetate (IIb) obtained from the periodic acid oxidation of elatericin A diacetate (Ib). Such transformation is convincing evidence for the location of the secondary hydroxyl group in carbon 2 of ring A in elatericin A (Ia). Furthermore the treatment of VIb with *p*-toluenesulfonic acid resulted in the elimination of the acetoxy group at position 16 of ring D (VIIb) in a similar way described above for elatericin A.

We may now come to the problem of assigning the position of the remaining oxygen atom in the molecule. It has been previously reported[5] that elatericin A (Ia) possesses a saturated carbonyl group absorbing at 1696 cm.$^{-1}$, which is unreactive toward 2,4-dinitrophenylhydrazine and other carbonyl reagents, and could not be reduced by normal reducing agents. It was suggested there, that it should occupy a hindered position. Of the possible positions left for consideration in ring B and C, we favor C(11) rather than C(12) on spectroscopic grounds. The ultraviolet spectrum of IIIb has a maximum at 240 mμ (ϵ 9,000); in steroids a Δ^{16}-12,20-diketone system exhibits an abnormally low maximum at 230 mμ; therefore the Δ^{16}-11,20-diketone is favored.[17] However the position C(7) may also account for the hindered carbonyl.

Elatericin A and B also contain an inert double bond.[10] They gave positive reactions with tetranitromethane; further, the analyses of all the degradation products fitted an empirical formula with two hydrogen atoms less than reported.[5,12] This double bond was also indicated during the ozonoly-

(13) A. R. H. Cole, "Fortschr. Chem. Org. Naturstoffe," Vol. XIII, Springer Verlag, Vienna, 1956, p. 37.

(14) R. E. Marker and D. L. Turner, THIS JOURNAL, **62**, 2541 (1940).

(15) W. Rigby, *J. Chem. Soc.*, 793 (1951).

(16) (a) L. Dorfman, *Chem. Revs.*, **53**, 65 (1953); (b) **53**, 82 (1953).

(17) C. Djerassi, E. Batres, J. Romo and G. Rosenkranz, THIS JOURNAL, **74**, 3634 (1952), ref. 10.

sis of compound VIIIb (obtained by the selective reduction of the double bond conjugated to the carbonyl in IIIb). Having assumed that the hindered carbonyl group is in ring C, the double bond could then be placed only in ring B. In view of these results, a revision of the molecular formulas of elatericin A and B to $C_{30}H_{44}O_7$ and $C_{30}H_{42}O_7$, respectively, is necessary.[10]

Having tentatively located the oxygenated functions and the double bond in elatericin A, (Ia) let us consider now the structure of its periodic acid oxidation products. Two moles of the peracid are consumed, cleaving the side chain and the α-hydroxy ketone in ring A.

The product, 2,3-*seco*-hexanor-elatericin A (IX) was amorphous, it displayed acidic properties and had an aldehyde band (2730 cm.$^{-1}$) in the infrared. Heating its sodium salt in a saturated solution of sodium carbonate resulted in ring closure at the available activated methylene group at C(1), and elimination of C(2) with the elements of formaldehyde (isolated as formic acid). Thus, the resulting cyclized product $C_{23}H_{32}O_4$ contained a five-membered ring A having a carbonyl group band at 1740 cm.$^{-1}$ in the infrared; structure X is therefore proposed for this degradation product. Heating this substance (X) with *p*-toluenesulfonic acid in benzene solution induced the expected elimination of the hydroxyl group at C(16) in ring D, and resulted in the formation of a double bond conjugated to the ketone as shown in XI. This change was well characterized by spectroscopic evidence: infrared, 1742, 1692, 1660 and 1590 cm.$^{-1}$, the two latter absorptions being related to the α,β-unsaturated ketone, while the ultraviolet spectrum showed now a new and strong absorption at 240 mμ (ϵ 8500). No hydroxyl band was recorded for this compound. The same compound (XI) was also obtained when X was heated in aqueous methanolic alkali or with acid.

These results corroborate the suggested structure Ia for elatericin A and IVa for elatericin B.

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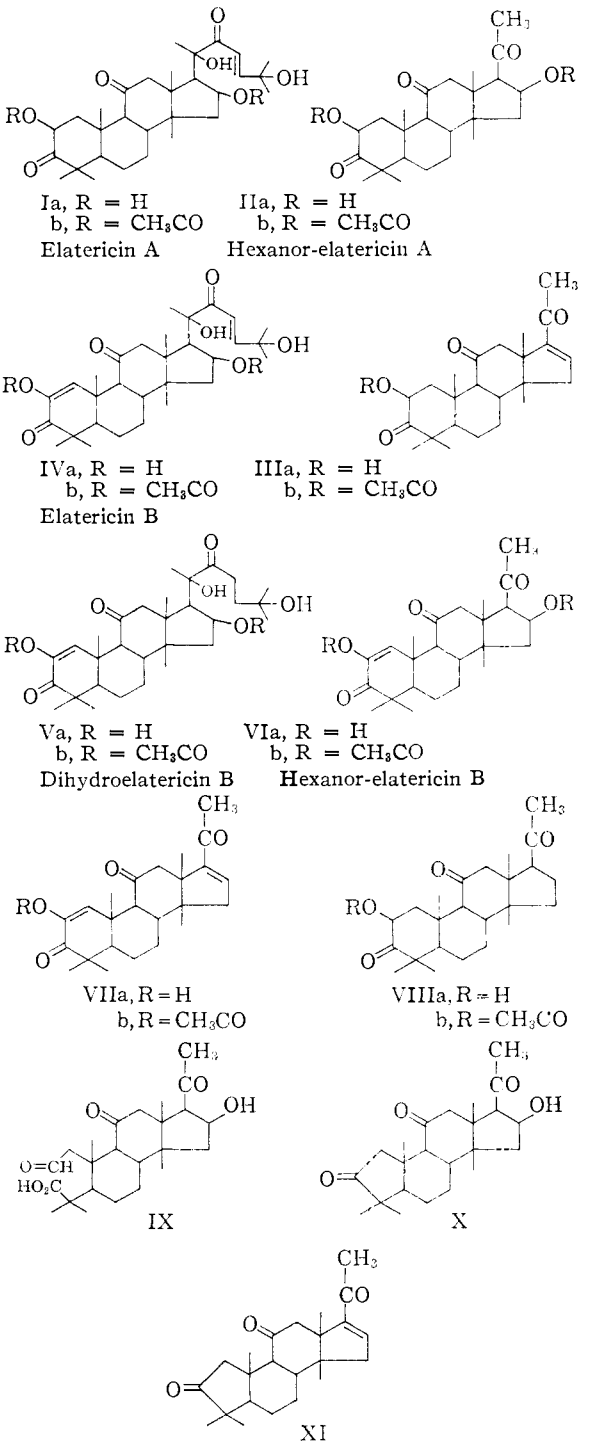
Experimental

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.

Oxidation of Elatericin A with Bismuth Oxide—Elatericin B (IVa).—A solution of elatericin A (1 g.) and bismuth oxide (1 g.) in glacial acetic acid (10 ml.) was refluxed for half an hour. The solution was cooled to room temperature and filtered from black bismuth and excess bismuth oxide; the filtrate was evaporated to dryness under reduced pressure. Chloroform was added to the residue and the resulting cloudy solution washed with dilute aqueous ammonia. The chloroform layer after separation was evaporated to dryness, and the residue dissolved in ether. The ethereal solution was shaken with cold 4% sodium hydroxide solution (3 × 20 ml.), and immediately separated, each portion being acidified without delay with cold dilute sulfuric acid. The combined acidified portions were extracted with ether, dried over sodium sulfate and evaporated to dryness. The residue crystallized from ethyl acetate–benzene, yield 250 mg., fine needles, m.p. 140–145°, $[\alpha]_D$ −44° in chf. (*c* 0.91).

CHART I[a]



[a] One inert double bond has been omitted purposefully and is assumed to be in ring B.

Further purification was obtained by dissolving the product in a solution of ethyl acetate–benzene (1:1) saturated with formamide and filtering through a column packed with a mixture of silicic acid and Celite (20 g., 1:1). The product was then crystallized twice from ethyl acetate–benzene, needles, m.p. 149–151°; a mixed m.p. with elatericin B gave no depression; $[\alpha]_D$ −51° in chf. (*c* 0.80); λ_{max} 230 mμ (ϵ 10,400) and 270 mμ (ϵ 5,000). The infrared spectrum was superimposable on that of an authentic sample of elatericin B. In ethanol, coloration was produced with ferric chloride.

Anal. Calcd. for $C_{30}H_{42}O_7$: C, 70.01; H, 8.23. Found: C, 69.72; H, 8.35.

The diacetate was prepared by acetylation overnight at room temperature in a solution of acetic anhydride–pyridine. The acetylation product crystallized from benzene–petroleum ether yielding elongated plates, m.p. 248–250° dec. $[\alpha]_D$ −82° in chf. (*c* 1.55), and was in all respects identical with an authentic sample of **elatericin B diacetate.**

Elatericin B Diacetate[7] (IVb).—Elatericin B (1 g.) from natural origin was acetylated overnight at room temperature in dry pyridine (10 ml.) and acetic anhydride (10 ml.). The mixture was decomposed with water and the solid filtered and washed with water. Several crystallizations from a mixture of benzene–petroleum ether yielded elongated plates (500 mg.), m.p. 249–251° dec., $[\alpha]_D$ −82° in chf. (*c* 1.52); negative ferric chloride test; λ_{max} 231 mμ (ε 20,500); ν_{max}^{KBr} 3500, 1757 (shoulder, enol acetate), 1738, 1692, 1637, 1240 (acetate) and 1200 (enol acetate) cm.$^{-1}$.

Anal. Calcd. for $C_{34}H_{46}O_9$: C, 68.20; H, 7.74; CH_3CO, 14.3. Found: C, 68.21; H, 7.75; CH_3CO, 15.2.

Oxidation of Elatericin A Diacetate with Periodic Acid, IIb.—A solution of periodic acid (3.5 g.) in water (40 ml.) was added to a solution of elatericin A diacetate (2.4 g.) in dioxane (50 ml.) and kept for 72 hours at room temperature. The mixture was then neutralized with a solution of sodium bicarbonate to *p*H 7, concentrated under reduced pressure and acidified with dilute sulfuric acid, and the solid which separated was extracted with chloroform. From the aqueous phase *trans*-4-hydroxy-4-methylpent-2-enoic acid was isolated. The chloroform phase was washed twice with a dilute solution of sodium bicarbonate and then with water. Drying and subsequent evaporation of the solution yielded 1.8 g. of an amorphous product.

This product was dissolved in 5 ml. of benzene, and chromatographed through a column packed with acid-washed alumina. Elution with benzene, followed by a mixture of ether–benzene (1:3) yielded small amounts of a non-crystalline material. The product obtained with the solvent mixture ether–benzene (1:1) was collected, and crystallized from ether; it yielded 600 mg. of **hexanor-elatericin A diacetate.** Repeated crystallizations from ether resulted in plates, m.p. 202–205°, $[\alpha]_D$ +112° in chf. (*c* 1.06); λ_{max} 288 mμ (ε 200); ν_{max} 1730 (broad), 1700 and 1240 cm.$^{-1}$, no hydroxyl band was recorded. The substance gave a positive iodoform test for a methyl ketone.

Anal. Calcd. $C_{28}H_{38}O_7$: C, 69.11; H, 7.87; CH_3CO, 17.7. Found: C, 69.31; H, 7.82; CH_3CO, 16.0.

Oxidation of Elatericin B Diacetate with Periodic Acid, VIb.—Elatericin B diacetate was oxidized with periodic acid as previously described for elatericin A diacetate. The resulting oxidation product, **hexanor-elatericin B diacetate** was purified by chromatography as reported and crystallized several times from ether–petroleum ether; needles, m.p. 182–184°, $[\alpha]_D$ +10° in chf. (*c* 1.44); λ_{max} 231 mμ (ε 10,000); ν_{max} 1757 (enol acetate), 1730, 1700, 1240 (acetate) and 1190 (enol acetate) cm.$^{-1}$, no hydroxyl band recorded. The substance gave a positive iodoform test for a methyl ketone.

Anal. Calcd. $C_{28}H_{36}O_7$: C, 69.40; H, 7.48; CH_3CO, 17.6. Found: C, 69.20; H, 7.70; CH_3CO, 18.9.

Deacetylation of IIb to Form IIIb.—To a solution of *p*-toluenesulfonic acid (150 mg.) in benzene (70 ml.), dried by azeotropic distillation, hexanor-elatericin A diacetate (IIb) (450 mg.) was added. The solution was heated under reflux for one hour, then cooled and washed with water to a neutral reaction. The organic layer was dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue crystallized from ether yielding 280 mg. of **deacetoxy-hexanor-elatericin A monoacetate (IIIb);** recrystallized twice from ether, plates, m.p. 237–245° dec., $[\alpha]_D$ +131° in chf. (*c* 1.36); λ_{max} 240 mμ (ε 9,000); ν_{max} 1735, 1728, 1695, 1660, 1590 and 1240 cm.$^{-1}$.

Anal. Calcd. $C_{26}H_{34}O_5$: C, 73.21; H, 8.04; CH_3CO, 10.0. Found: C, 73.25; H, 7.82; CH_3CO, 10.4.

Heating to reflux hexanor-elatericin A diacetate (IIb) in an aqueous methanolic solution of hydrochloric acid (5%) for one hour resulted in an amorphous product. Acetylation of this product at room temperature with acetic anhydride–dry pyridine yielded substance IIIb, m.p. and mixed m.p. 237–245° dec. $[\alpha]_D$ + 130° in chf. (*c* 1.32); same infrared spectrum throughout the whole range.

Anal. Calcd. $C_{26}H_{34}O_5$: C, 73.21; H, 8.04. Found: C, 72.87; H, 8.30.

Deacetylation of VIb to Form VIIb.—To a solution of *p*-toluene sulfonic acid (100 mg.) in benzene (70 ml.) dried by azeotropic distillation, hexanor-elatericin B diacetate (VIb) (380 mg.) was added. The solution was heated and washed as described above, and the reaction product dissolved in benzene and chromatographed through a column packed with acid-washed alumina (20 g.). Benzene (200 ml.) and a mixture of ether–benzene (1:9, 200 ml.) were passed through the column and the oily amorphous product obtained was rejected; a mixture of ether–benzene (2:8) eluted the major part of the reaction product, **deacetoxyhexanor-elatericin B monoacetate (VIIb),** which was crystallized twice from ether; plates, m.p. 238–240° dec., $[\alpha]_D$ + 11° in chf. (*c* 2.0); λ_{max} 237 mμ (ε 19,000); ν_{max} 1757 (enol acetate), 1693, 1663, 1585 and 1185 (enol acetate) cm.$^{-1}$.

Anal. Calcd. $C_{26}H_{32}O_5$: C, 73.56; H, 7.60. Found: C, 73.53; H, 7.68.

Reduction of VIb to IIb.—Hexanor-elatericin B diacetate (VIb, 250 mg.) in alcohol (30 ml.) was hydrogenated over palladium-on-charcoal 10% at room temperature and atmospheric pressure. The hydrogenation ceased when 11.5 ml. of hydrogen was absorbed (calculated for one double bond 12.3 ml.). The catalyst was filtered and the solvent evaporated to dryness. The residue was dissolved in benzene (3 ml.) and chromatographed on acid-washed alumina (25 g.). The fractions eluted with benzene (250 ml.) and a mixture of ether–benzene (1:9, 250 ml.) were rejected. The product eluted with the solvent mixture ether–benzene (1:4) was collected and crystallized twice from ether; yield 100 mg., plates, m.p. 203–205°, $[\alpha]_D$ + 110° in chf. (*c* 1.02). A mixed m.p. with **hexanor-elatericin A diacetate** (IIb) gave no depression; the infrared spectra of both compounds were found to be identical throughout the whole range.

Anal. Calcd. $C_{28}H_{38}O_7$: C, 69.11; H, 7.87. Found: C, 69.14; H, 7.84.

A(2)-Nor-hexanor-elatericin A (X).—To periodic acid (4.8 g.) dissolved in water (125 ml.), a solution of elatericin A (3 g.) in dioxane (80 ml.) was added, and the mixture kept at room temperature for 48 hours. The homogeneous solution was then neutralized to *p*H 7 with a saturated solution of sodium carbonate, and concentrated to a small volume under reduced pressure. Upon acidification of the solution with dilute sulfuric acid an amorphous precipitate was obtained and extracted with ether (500 ml.). The organic layer was then shaken with a saturated solution of sodium carbonate (3 × 70 ml.). The carbonate solution was filtered and reacidified with sulfuric acid. Extraction with ether, drying over sodium sulfate and evaporation of the solvent left an amorphous residue (2.2 g.) which could not be induced to crystallize. In addition to the carboxyl group, the presence of an aldehyde group was indicated by the infrared spectrum: ν_{max} 2730 cm.,$^{-1}$ and a positive dimedone test: **2,3-seco-hexanor-elatericin A (IX).**

The above product IX was dissolved in a saturated solution of sodium carbonate (250 ml.) and heated on a steam-bath for two hours, whereupon a crystalline product separated. The crystals were filtered and washed with water; yield 1.5 g. Repeated crystallizations from chloroform–petroleum ether followed by crystallizations from absolute alcohol yielded plates, m.p. 210–213° (sublimes upon melting), $[\alpha]_D$ + 66° in chf. (*c* 1.65), no major absorption in the ultraviolet spectrum; ν_{max} 3425, 1740 (five-membered ring ketone), 1695 cm.$^{-1}$. The substance gave a positive iodoform test for a methyl ketone.

Anal. Calcd. $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.04; H, 8.67.

Identification of Formic Acid in the Reaction Mixture of X.—The sodium carbonate solution filtrate, obtained from the separation of X, was carefully acidified with dilute sulfuric acid and steam distilled. The distillate which gave an acidic reaction was collected until neutral reaction was reached (250 ml.; 1 ml. titrated 0.75 ml. of a 0.01 *N* sodium hydroxide solution). The presence of formic acid in this solution was indicated by its reduction to formaldehyde which was identified by a positive chromotropic acid test.[18] A blank reaction using the same set of reagents without elatericin A showed no formation of formic acid.

(18) F. Feigl, "Spot Tests," Vol. II, Elsevier Publishing Co., Houston, Tex., 1954, p. 245.

Δ^{16}-Desoxy-A(2)-nor-hexanor-elatericin A (XI).—To a solution of *p*-toluenesulfonic acid (200 mg.) in benzene (200 ml.) dried by azeotropic distillation, compound X (650 mg.) was added. The resulting homogeneous solution was refluxed for one hour, while the water–benzene azeotrope was continuously removed. The solution was then cooled to room temperature and washed with water until the washings had neutral reaction. The benzene layer was dried over sodium sulfate and evaporated to dryness. The crystalline residue was dissolved in benzene, and chromatographed on acid-washed alumina (50 g.). The following solvents were passed successively through the column: benzene (400 ml.), ether–benzene (1:9, 300 ml.), ether–benzene (1:4, 300 ml.). A crystalline product finally emerged with a solution of ether–benzene (1:1). Crystallization from ether–petroleum ether gave prisms, 400 mg., m.p. 197–199°, $[\alpha]D + 28°$ in chf. (*c* 2.15), λ_{max} 240 mμ (ε 8,500); ν_{max}1742 (five-membered ring ketone), 1692 (hindered ketone), 1660[13] (Δ^{16}-20-ketone) and 1590 (Δ^{16}-double bond) cm.$^{-1}$. The product deteriorates upon standing.

The same dehydration of compound X could be accomplished by heating an aqueous methanol solution of the compound in the presence of acid or alkali.

Anal. Calcd. $C_{23}H_{30}O_3$: C, 70.01; H, 8.23. Found: C, 69.72; H, 8.35.

Dihydro-elatericin B Diacetate (Vb).—Elatericin B diacetate (43 mg.) was reduced in ethanol over palladium-on-charcoal 5%; the calculated amount of 1.7 ml. of hydrogen was rapidly absorbed. The catalyst was filtered and the solvent evaporated to dryness. The amorphous residue could not be induced to crystallize; λ_{max} 231 mμ (ε 7,000) for enol acetate; ν_{max}^{KBr} 3500, 1757 (enol acetate), 1738 (acetate), 1700 (broad), 1240 (acetate) and 1200 (enol acetate) cm.$^{-1}$.

The same compound could be obtained by the acetylation of dihydro-elatericin B with acetic anhydride and pyridine at room temperature.

Dehydrogenation of Elatericin A.—Elatericin A was reduced over palladium-on-charcoal 10% in ethanol solution to hexahydro-elatericin A[5] ν_{max} 1693 cm.$^{-1}$ (for hindered ketone). Hexahydro-elatericin A (2 g.) was further reduced by stirring and refluxing with lithium aluminum hydride (2 g.) in tetrahydrofuran (500 ml.) during 70 hours. The mixture was decomposed with a saturated solution of sodium sulfate and 100 ml. of ethyl acetate was added. The insoluble salts were filtered and the filtrate dried over sodium sulfate and evaporated to dryness. The amorphous residue did not show any carbonyl absorption in the infrared.

Of the above reduced product, 6 g. was thoroughly mixed with 12 g. of selenium powder and heated under a stream of nitrogen at 340–360° during 50 hours. The cooled solid was finely ground and extracted in a soxhlet with pentane during 24 hours. Evaporation of the solvent left 1.2 g. of a viscous black oil which was distilled *in vacuo*. Three fractions were collected: fract. 1, 0.13 g., b.p. 37–41° (0.5 mm.); fract. 2, 0.8 g., b.p. 100–130° (0.4 mm.); fract. 3, 0.57 g., b.p. 130–170° (0.4 mm.). Only fract. 2 was found to be devoid of any carbonyl absorption in the infrared and was chromatographed on alumina (80 g.) using pentane as solvent. With this solvent an oil was obtained which did not possess any characteristic aromatic spectrum in the ultraviolet. No adducts were obtained with 1,3,5-trinitrobenzene. Further development of the column with a mixture of pentane–benzene (9:1) yielded 90 mg. of a yellow oil. This oil was rechromatographed on alumina using the same solvents sequence, and 14 mg. of a semi-solid product which could not be induced to crystallize was obtained. The ultraviolet spectrum of this product in isopropyl alcohol solution showed the following absorptions: λ_{max} 262, 282, 293, 306 and 338 mμ. The location and the relative intensity of these absorption maxima corresponded to the ultraviolet spectrum of **1,2,8-trimethylphenanthrene**. In alcoholic solution this product gave an adduct with 1,3,5-trinitrobenzene; yellow needles, m.p. 174–178°; mixed m.p. with an authentic sample of an 1,3,5-trinitrobenzoate adduct of 1,2,8-trimethyl phenanthrene gave no depression.

Further development of the column with more polar solvents did not yield any aromatic products.

REHOVOTH, ISRAEL

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, NEW YORK UNIVERSITY COLLEGE OF MEDICINE]

Synthesis of Cytidine 5′-Diphosphate and Guanosine 5′-Diphosphate[1]

BY ROBERT WARNER CHAMBERS, PHILIP SHAPIRO[2] AND VIKTOR KURKOV

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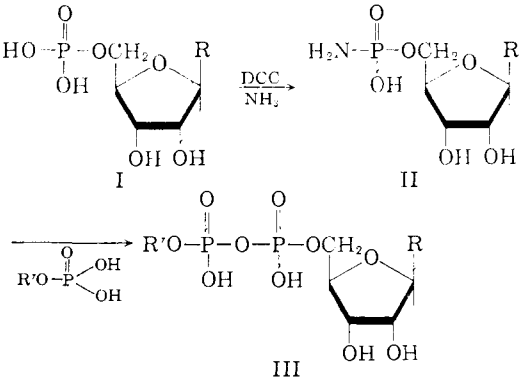
The preparation of two new nucleoside 5′-phosphoramidates, cytidine 5′-phosphoramidate and guanosine 5′-phosphoramidate, is described. The synthesis of the corresponding nucleoside 5′-diphosphates in good yield by a reaction between the nucleoside phosphoramidate and phosphoric acid is reported and certain important aspects of this general reaction are discussed. Studies on the preparation of cytidine 5′-phosphate and an improved synthesis of acetone cytidine are described.

It has now been established that several unsymmetrical pyrophosphates (III) of biological interest



can be prepared in good yield by a simple two-step synthesis that utilizes a nucleoside 5′-phosphoramidate (II) as the key intermediate.

In a previous communication,[3] we reported the synthesis of adenosine 5′-phosphoramidate (II, R = adenine) and uridine 5′-phosphoramidate (II, R = uracil) by reacting the corresponding nucleotide I with dicyclohexylcarbodiimide (DCC) and ammonia. The general usefulness of these intermediates has been amply demonstrated by the synthesis of adenosine 5′-diphosphate[4] (III, R = adenine, R′ = H,ADP), uridine 5′-diphosphate[5] (III, R = uracil, R′ = H,UDP), uridine diphosphate glucose[6] (III, R = uracil, R′ = glucose) and flavin adenine dinucleotide[6] (III, R = adenine, R′ = riboflavin). Two new nucleoside 5′-phos-

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(2) Summer Research Fellow, supported by a grant from the United States Public Health Service.

(3) R. W. Chambers and J. G. Moffatt, THIS JOURNAL, **80**, 3752 (1958).

(4) R. W. Chambers and H. G. Khorana, *ibid.*, **80**, 3749 (1958).

(5) R. W. Chambers, *ibid.*, **81**, 3022 (1959).

(6) J. G. Moffatt and H. G. Khorana, *ibid.*, **80**, 3756 (1958).