mixture was shaken in a hydrogen atmosphere for 5–15 minutes. The calculated amount of hydrogen had then been absorbed. The catalyst was filtered off, the filtrate concentrated to about 1 ml. at reduced pressure, and the concentrate which contains large amount of crystals allowed to stand in an ice-box to complete crystallization. The yield after filtering and washing with methanol was 0.39 g. (91%). The product was purified by recrystallization from 20–40% aqueous ethanol and was obtained as colorless plates, m.p. 221.5–222.5° dec. For analysis the crystals were dried at 0.1–0.2 mm. and 100° for one hour.

Anal. Calcd. for $C_{11}H_{14}O_5N_2$: C, 51.97; H, 5.51; N, 11.03. Found: C, 51.80; H, 5.67; N, 11.06.

N-(3-Aminosalicyloyl)-L-threonine (L-Antimycic Acid) (VIb).—An amount of 1.1 g. of N-(3-nitro-2-benzyloxybenzoyl)-L-threonine (IIIb) was reduced catalytically with 0.7 g. of palladium-on-charcoal (5%) in 70 ml. of methanol solution by the above method, and the product was obtained in 84% (0.52 g.) yield. Recrystallization from 20–40% aqueous ethanol gave colorless plates, m.p. 224–225° dec., $[\alpha]^{17}D$ + 15° (*c* 1.36, 3% hydrochloric acid). The mixed melting point of this product with natural antimycic acid[4] showed no depression. For analysis the crystals were dried at 0.1–0.2 mm. and 100° for one hour.

Anal. Calcd. for $C_{11}H_{14}O_5N_2$: C, 51.97; H, 5.51; N, 11.03. Found: C, 51.67; H, 5.69; N, 11.17.

N-(3-Aminosalicyloyl)-DL-threonine-Methyl Ester-Methyl Ether (DL-Antimycic Acid-Methyl Ester-Methyl Ether) (VIIa).—When 104 mg. of N-(3-aminosalicyloyl)-DL-threonine (VIa) was methylated with diazomethane by the procedure used for preparation of natural antimycic acid-methyl ester-methyl ether,[1] the yield was 40 mg. (36%). Recrystallization from ethyl acetate gave colorless needles, m.p. 127.5–129°. For analysis the crystal were dried in a vacuum at 80° for one hour. This product did not depress the melting point of N-(3-amino-2-methoxybenzoyl)-DL-threonine-methyl ester prepared from N-(3-amino-2-methoxybenzoyl)-DL-threonine.[1]

Anal. Calcd. for $C_{13}H_{18}O_5N_2$: N, 9.93. Found: N, 9.86.

N-(3-Aminosalicyloyl)-L-threonine-Methyl Ester-Methyl Ether (L-Antimycic Acid-Methyl Ester-Methyl Ether) (VIIb).—A yield of 50 mg. (43%) of VIIb was obtained by the above method from 100 mg. of N-(3-aminosalicyloyl)-L-threonine (VIb). Recrystallization from ethyl acetate gave colorless needles of m.p. 155–156°, which were identical with natural antimycic acid-methyl ester-methyl ether. For analysis the crystals were dried in a vacuum at 80° for one hour.

Anal. Calcd. for $C_{13}H_{18}O_5N_2$: N, 9.93. Found: N, 10.02.

N-(3-Acetaminoacetylsalicyloyl)-α-aminocrotonic Azlactone (Antimycic Acid Diacetate) (VIII).—(a) A mixture of 45 mg. of N-(3-aminosalicyloyl)-DL-threonine (VIa), 0.7 ml. of acetic anhydride and 2 drops of pyridine was heated for 3 minutes at 95–100°. After cooling, the crystals were filtered off and washed with acetic anhydride (yield 43 mg., 80%). Recrystallization from acetic anhydride gave fibrous needles, m.p. 210–211° dec.

Anal. Calcd. for $C_{15}H_{14}O_5N_2$: N, 9.27. Found: N, 9.48.

(b) Reaction of 80 mg. of N-(3-aminosalicyloyl)-L-threonine (VIb) with 1.5 ml. of acetic anhydride and 3 drops of pyridine as above gave the same azlactone (yield 86 mg., 90%). This product upon recrystallization from acetic anhydride gave fibrous needles, m.p. 210–211° dec. The mixed melting point with natural antimycic acid diacetate[4,5] showed no depression.

Anal. Calcd. for $C_{15}H_{14}O_5N_2$: N, 9.27. Found: N, 9.22.

N-(3-Acetaminosalicyloyl)-α-aminocrotonic Acid (IX).—A mixture of 85 mg. of N-(3-acetaminoacetylsalicyloyl)-α-aminocrotonic azlactone (VIII) and 7 ml. of 0.1 *N* aqueous sodium hydroxide was stirred at room temperature (15–20°) until the solution became clear. When the solution was adjusted to about *p*H 1 with hydrochloric acid, colorless plates were deposited. The yield after filtering and washing with water was 73 mg. (93%). Recrystallization from 50% aqueous ethanol or ethanol gave colorless plates or fine needles, m.p. 220–221° dec. For analysis the crystals were dried at 0.1–0.2 mm. and 100° for one hour.

Anal. Calcd. for $C_{13}H_{14}O_5N_2$: N, 10.07. Found: N, 10.20.

Acknowledgments.—The authors wish to express their sincere appreciation to Prof. F. M. Strong of the University of Wisconsin for his interest, and his helpful comments made in connection with the preparation of this manuscript; and to the Tanabe Pharmaceutical Company for its support. Thanks are due to Miss Teruko Ueda of this Laboratory for the microanalyses.

(5) Natural antimycic acid diacetate described by Tener, *et al.*,[4] melted at 202–202.5°, but after recrystallization from acetic anhydride the melting point was raised to 210–211°.

TOKUSHIMA, JAPAN

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF BOSTON UNIVERSITY]

Location of Ketone and Hydroxyl Functions of Cassaic Acid[1]

BY WALTER J. GENSLER AND GWENDOLYN M. SHERMAN

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Methyllithium reacts with the acetyl derivative of decarboxylated cassaic acid to furnish a product that can be aromatized to 1,7,8,9-tetramethylphenanthrene. Methylmagnesium iodide adds to only one of the two ketone groups in decarboxylated diketocassenic acid. The addition product after lithium aluminum hydride reduction and selenium aromatization is converted to 1,2,7,8-tetramethylphenanthrene. The work establishes the correctness of the earlier, provisionally assigned, positions for the hydroxyl and the ketone groups of cassaic acid.

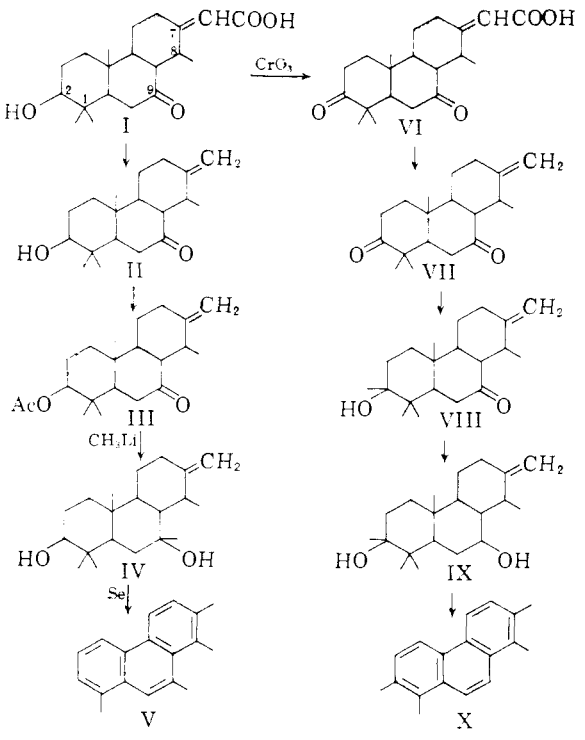
Formulation I, advanced by Humber and Taylor[2a] and by Tondeur,[2b] is consonant with the observed chemistry of cassaic acid.[3] Although the carbon skeleton has been established,[4] the locations of the hydroxyl and ketone groups have remained unproved. We are now reporting evidence in support of the provisional assignments of position 2 for the hydroxyl and position 9 for the ketone group. Our plan of attack, as discussed below, called for the use of methyl groups as markers for the oxygen functions.

(1) Abstracted from the Dissertation submitted by Gwendolyn M. Sherman to the Graduate School of Boston University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, 1959.

(2) (a) L. G. Humber and W. I. Taylor, *J. Chem. Soc.*, 1044 (1955); (b) R. Tondeur, Ph.D. Thesis, E.T.H., Zurich, 1950.

(3) *Cf.* G. Dalma in Chapter 36 of "The Alkaloids," by R. H. F. Manske and H. L. Holmes, Vol. IV, Academic Press, Inc., New York, N. Y., 1954; T. A. Henry, "The Plant Alkaloids," 4th edition, Blakiston Co., Philadelphia, Pa., 1949, p. 725.

(4) See F. E. King, T. J. King and J. M. Uprichard, *J. Chem. Soc.*, 3428 (1958).



The projected addition of methylmagnesium iodide to the ring ketonic group of cassaic acid (I) was complicated by the presence of the carboxyl group. Conversion of cassaic acid, by decarboxylation, to the corresponding 7-methylene compound II disposed of this problem. However, another difficulty arose when it was found that the methyl Grignard reagent was reluctant to add to the ketone function. Infrared absorption curves of products from Grignard addition experiments with acetyl derivative III, by showing intense 5.88 μ absorption, indicated that the ketone group did not react. The same was found to be true in methyl Grignard reactions with cassaic acid (I), with the methyl ester of cassaic acid, or with the acetyl derivative of the parent alkaloid, cassaine.[3] The reaction of hydroxy ketone II with methylenetriphenylphosphine[5] taken in large excess again failed to give a carbonyl-free product.[6]

The explanation for this behavior could be steric in nature, since the axial 8-methyl group[6a] introduces an element of hindrance toward reactions at position 9. However this hindrance is clearly borderline, inasmuch as methyllithium (*cf.* below) and methylmagnesium under forcing conditions[6] can add to the ketone group, and other ketone reactions[3] have been realized. Another factor in the difficult Grignard additions is suggested by the results of Zerewitinoff determinations, which in several instances indicated active C–H in the molecule. If so, Grignard enolate formation involving hydrogen at the ketone α-positions could block Grignard addition.

Fortunately, addition of methyllithium to the ketonic group of compound III, although still difficult, proved to be feasible. When the resulting dihydroxy derivative IV was aromatized with selenium, 1,7,8,9-tetramethylphenanthrene (V) was obtained. Direct comparisons with authenic 1,7,8,9-tetramethylphenanthrene synthesized for this purpose[7] left no doubt as to the identity of the degradation product V.

Earlier work had shown that 1,7,8-trimethylphenanthrene emerged as the selenium aromatization product from several cassaic acid derivatives, *viz.*, dihydroxycassanic acid, methyl dihydrocoumingidate and cassanic acid.[8] Accordingly, appearance of a fourth methyl group at position 9 of the phenanthrene product from sequence I–V served to demonstrate the presence of a carbonyl function at position 9 of cassaic acid (I).

In order to label the hydroxyl position of cassaic acid with methyl, the hydroxyl had to be converted to carbonyl. The reaction path that was worked out started with diketocassenic acid (VI),[9] which could be obtained from cassaic acid by chromic anhydride oxidation. Decarboxylation furnished diketone VII, in which it was anticipated that the ketone at the 9-position would resist addition of the methyl Grignard reagent. This was found to be the case when treatment of diketone VII with methylmagnesium iodide in excess produced hydroxy ketone VIII. Reduction to the corresponding dihydroxy derivative IX followed by selenium aromatization gave 1,2,7,8-tetramethylphenanthrene (X). The identity of this substance was established by direct melting point comparisons with authentic synthetic material[10] provided by Dr. B. G. Engel, as well as by comparison of infrared and ultraviolet absorption spectra.

Isolation of 1,2,7,8-tetramethylphenanthrene showed that a carbonyl group must be present at

(5) Compare F. Sondheimer and R. Mechoulam, THIS JOURNAL, **79**, 5029 (1957).

(6) Professor R. B. Turner has called our attention to a monograph (A. Ronco, "Zur Kenntnis der Erythrophleum Alkaloide: Ueber die Konstitution der Cassainsäure," Kommerzdruck und Verlag, AG, Zurich, 1945) in which the reaction of methylmagnesium bromide with acetoxyketocassanic methyl ester under forcing conditions is described. When the reaction was allowed to proceed for fifteen hours with boiling toluene as solvent, the ester *and* ketone function were considered to react, so that three methyl groups were introduced. One of the two products isolated was thought to be a saturated triol, $C_{23}H_{42}O_3$ (or possibly(?) $C_{23}H_{40}O_3$); the second product was thought to be an unsaturated diol, $C_{23}H_{40}O_2$, derived by elimination of water from the triol. However, these molecular formulas and structural inferences depend almost entirely on the results of C and H analyses. Since the C and H content of the dihydroxy ketone, $C_{22}H_{38}O_3$, and of the corresponding unsaturated hydroxy ketone, $C_{22}H_{36}O_2$, resulting from action *only* at the ester function do not differ enough from the experimental values to preclude these latter structures, it is possible that the ketone group did *not* react even under forcing conditions. The figures for the "saturated triol" are—Calcd. for $C_{23}H_{42}O_3$: C, 75.36; H, 11.55. Calcd. for $C_{23}H_{40}O_3$(?): C, 75.77; H, 11.06. Calcd. for $C_{22}H_{38}O_3$: C, 75.38; H, 10.93. Found: C, 75.80; H, 10.99. The figures for the "unsaturated diol" are—Calcd. for $C_{23}H_{40}O_2$: C, 79.25; H, 11.57. Calcd. for $C_{22}H_{36}O_2$: C, 79.46; H, 10.92. Found: C, 79.15; H, 11.59.

(6a) An account of the work leading to this conformational assignment as well as of other successful work on total synthesis was presented on November 6–7, 1958, at the Quartermaster Research and Development Center, Natick, Massachusetts, and will be published in "Tetrahedron Letters" under the authorship of R. B. Turner, R. G. Herzog, R. B. Morin and A. Riebel. We wish to thank Professor Turner for providing us with a copy of his paper in manuscript form as well as for information about Ronco's work.[6]

(7) Ph.D. Dissertation of S. C. Chakravarti, 1959.

(8) L. Ruzicka and G. Dalma, *Helv. Chim. Acta*, **22**, 1516 (1939); E. Schlittler, *ibid.*, **24**, 319E (1941); L. Ruzicka, G. Dalma and W. B. Scott, *ibid.*, **24**, 179E (1941).

(9) G. Dalma, *ibid.*, **22**, 1497 (1939).

(10) B. G. Engel, A. Ronco, K. Berse, Pl. A. Plattner and L. Ruzicka, *ibid.*, **32**, 1713 (1949).

position 2 of diketones VII and VI, and that either hydroxyl or ketone must be present at position 2 of cassaic acid (I). However, since the ketone of cassaic acid was fixed at position 9, the original hydroxyl group must be at position 2.[11] It is noteworthy that cassaic acid, which has no carbonyl function at position 2, gave no color in the Zimmermann test, whereas diketocassenic acid, which does have a carbonyl at position 2, did give a positive test. This behavior is consistent with the report that the Zimmermann test is positive for triterpenes containing a carbonyl group at the 3-position (steroid numbering) but is negative when the carbonyl group is at other positions.[12]

The presently determined positions of hydroxyl and ketone groups are in accord with the degradative and the total synthesis work of Turner and his co-workers.[6a] These positions also have been shown to be correct by Dr. D. W. Mathieson and his students (private communication).

Acknowledgment.—We wish to thank Dr. Bruno G. Engel for a sample of synthetic 1,2,7,8-tetramethylphenanthrene, and to thank Mr. A. D. Lewis and Dr. John A. King of Warner–Chilcott Laboratories for a supply of cassaine bisulfate. The Director General of Institut National pour l'Etude Agronomique du Congo Belge, through the good offices of Mr. R. G. McGregor, American Consul in Leopoldville, and Dr. W. O. Brown, Director, African Research and Studies Program at Boston University, provided us with some of the *Erythrophleum guineense* bark used in this work. S. B. Penick and Co., New York, coöperated in several ways. Mr. E. Meier of the Weizmann Institute of Science very kindly determined several active hydrogen values, and Dr. E. R. Blout of Polaroid Corporation very kindly arranged for the determination of two of the optical rotations. The work was supported in part by a Public Health Service (National Heart Institute) predoctoral fellowship (1955–1958) to G.M.S., for which we are grateful.

Experimental[13]

Cassaic Acid (I).—Cassaine bisulfate was isolated from powdered bark of *Erythrophleum guineense* essentially as described before.[9,14] When sealed in an evacuated capillary, the crystalline salt melted with slight discoloration at 305–306° dec. The melting point determined in the ordinary way was 292–293° dec. The optical rotation was $[\alpha]^{24}D$ −86 ±2° (1% in 0.1 *N* sulfuric acid). The constants reported before[9,14] are: m.p. *in vacuo*, 290° dec., and $[\alpha]^{23}D$ −95 ± 2° (*c* 1 in 0.1 *N* sulfuric acid). Cassaine base obtained from the bisulfate showed m.p. 139.5–140°, optical rotation $[\alpha]^{22}D$ −101 ± 2° (1.5% in absolute alcohol), and an ultraviolet absorption maximum at 222 mμ (log ε 4.23) in 3.74×10^{-5} *M* absolute alcohol solution. Cassaine has been reported before[9,14,15] with m.p. 141° and 142.5°, $[\alpha]^{20}D$ −104.2° (*c* 2.044 in 96% alcohol), $[\alpha]^{20}D$ −111° (*c* 1 in 95% alcohol), $[\alpha]^{23}D$ −110.5 ± 2° (*c* 1 in 95% alcohol), $[\alpha]^{20}D$ −103° (*c* 1 in absolute alcohol), and λ_{max} 223 mμ (log ε 4.26) in alcohol.[16] As active hydrogen determination on cassaine[17] showed 0.38% as compared with the calculated value of 0.25% for one active hydrogen.

Acid hydrolysis[9] of cassaine to cassaic acid (I) was effected by heating a mixture of 0.76 g. of cassaine in 35 ml. of 1 *N* hydrochloric acid for 4 hours. After standing overnight the mixture was filtered. The yellow crystalline product was washed on the funnel with cold distilled water and was dried *in vacuo*. This material (0.51 g., m.p. 204.5–209.5°) was purified by recrystallization from aqueous acetone to give cassaic acid, m.p. 205.5–207°, $[\alpha]^{20}D$ −120° (1% in 95% alcohol), and λ_{max} 219 mμ (log ε 4.1) in 3.0×10^{-5} *M* absolute alcohol.

Anal. Calcd. for $C_{30}H_{50}O_4$: C, 71.82; H, 9.04. Found: C, 71.7; H, 9.0.

Cassaic acid as a mull with mineral oil showed infrared absorption maxima at 2.85, 5.85, 5.97 and 6.13 μ. The reported constants are m.p. 203°, $[\alpha]^{20}D$ −126.3° (*c* 1 in 95% alcohol), $[\alpha]^{20}D$ −111.6° (2.044 in acetone)[9,15] and λ_{max} 215 mμ (log ε 4.3) in alcohol.

Cassaic acid could be obtained conveniently by substituting cassaine bisulfate for cassaine in the above procedure. Cassaic acid could also be formed by saponification of cassaine. No allocassaic acid was encountered.

Decarboxylation of Cassaic Acid (I).—A mixture of cassaic acid (195 mg.) with 2 ml. of quinoline that had been distilled from zinc dust was boiled under a nitrogen atmosphere for 7 hours. The cooled solution, diluted with 25 ml. of ether, was washed with three 15-ml. portions of 2 *N* hydrochloric acid, then with water, with two 10-ml. portions of 10% sodium hydroxide solution, with two portions of water, and finally with saturated sodium chloride solution. The solution was filtered through a bed of anhydrous sodium sulfate, and all solvent was removed at 100°. The residue of crude decarboxylation product II weighed 146 mg., and showed infrared absorption peaks (as a non-crystalline film) at 2.86, 5.87, 6.04 and 11.15 μ.

Decarboxylation was also possible by heating cassaic acid alone for 75 minutes at 254° in a nitrogen atmosphere. The product was acetylated directly and processed essentially as described below. The acetyl derivative from the decarboxylation product obtained in the absence of quinoline could not be induced to crystallize.

Acetyl Derivative III of Decarboxylated Cassaic Acid.—The decarboxylated product (146 mg.) was heated on the steam-bath together with 4 ml. of pure benzene and 1 ml. of pure acetic anhydride for 20 hours. Material volatile at steam temperatures and water-pump pressures was removed, and the solid residue was dried in a vacuum desiccator over calcium chloride. A solution of the solids (145 mg.) in a small volume of 1:1 benzene–(30–60°) petroleum ether was passed through a short chromatography column made up of 0.75 g. of Merck acid-washed alumina. Additional 1:1 benzene–petroleum ether was used for elution, eluate being collected in 8-ml. fractions. Removal of solvent from the first two fractions gave 114 and 8 mg., respectively, of crystalline product. The combined materials after recrystallization from aqueous methanol weighed 83 mg. and melted at 116–117.5°. Another crystallization from aqueous acetone brought the melting point of the acetylated decarboxylated cassaic acid (III) to a constant value of 123–124.5°; the sample prepared for analysis by a further crystallization and dried showed m.p. 123–124°.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.90; H, 9.70.

A Zerewitinoff determination was performed on a sample dried at 56° over phosphorus pentoxide for 3 days. Methane corresponding to 0.14% of active hydrogen was evolved either at 0 or at 100° in anisole solvent. The value calculated for one active hydrogen is 0.30%. Active hydrogen determination using lithium aluminum hydride at room temperature showed 0.24%. Acetylated decarboxylated cassaic acid (III), $[\alpha]^{25}D$ −30° (0.19% in absolute ethanol), showed no ultraviolet absorption maxima at 210–320 mμ in 4×10^{-5} *M* alcohol solution. As a mineral oil mull, derivative III showed infrared absorption peaks at 5.80, 5.88, 6.06(w) and 11.14 μ. As a 1% solution in carbon tetrachloride the compound showed infrared absorption peaks at 3.26, 5.75, 5.86, 6.06 and 11.15 μ.

1,7,8,9-Tetramethylphenanthrene (V) from Acetoxy Ketone III.—A. Methyllithium with Compound III.—To

(11) A preliminary note dealing with the position of the hydroxyl group has appeared in *Chemistry & Industry*, 223 (1959).

(12) D. H. R. Barton and P. De Mayo, *J. Chem. Soc.*, 887 (1954).

(13) Melting points are uncorrected. Analyses were performed by Carol K. Fitz, Needham Heights, Mass., by Stephen M. Nagy, Microchemical Laboratory, Massachusetts Institute of Technology, Cambridge, and by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

(14) B. G. Engel and R. Tondeur, *Helv. Chim. Acta*, **32**, 2364 (1949).

(15) F. Faltis and L. Holzinger, *Ber.*, **72**, 1443 (1939).

(16) L. Ruzicka and G. Dalma, *Helv. Chim. Acta*, **22**, 1516 (1939).

(17) Private communication from Dr. B. G. Engel.

an ice-cold solution of 100 mg. (0.3 millimole) of keto acetate III dissolved in 1.5 ml. of dry ether was added 1.4 ml. of ethereal methyllithium (3.1 millimoles or approximately 10 molar proportions). The ethereal methyllithium was transferred with a syringe. The heavy white precipitate that had formed on adding the reagent dissolved in a short time. The slightly cloudy solution was kept cold for 1 hour and then allowed to stand at room temperature for 18 hours. After a final half-hour reflux period, the cooled mixture was treated with approximately an equal volume of saturated aqueous ammonium chloride solution. The ether layer was washed with two portions of water, and with saturated sodium chloride solution, then filtered through anhydrous sodium sulfate, and evaporated. The residue (87 mg.) consisted of a mixture of white crystals and yellow oil. The infrared absorption curve for this material showed two peaks in the hydroxyl region. The 5.88 μ carbonyl peak, although present, was greatly reduced in intensity.

This residue combined with an additional quantity of 130 mg. of keto acetate III was dissolved in a small volume of dry ether, and was again treated with ethereal methyllithium in tenfold molar excess. The reaction was carried out just as described in the preceding paragraph except that a magnetic stirrer was utilized, and the final reflux period was extended to 3 hours. A weak, although definite, infrared carbonyl absorption showed that the product (181 mg.) from this reaction, as in the first reaction, still contained ketonic material.

The product dissolved in a small volume of anhydrous 2:1 benzene–(30–60°) petroleum ether was placed on a 13 × 35 mm. column of Fisher alumina (4 g.). Eluate made up of the following solvents was collected in 7–8-ml. fractions: 2:1 benzene–petroleum ether (25 ml.), 9:1 benzene–ether (45 ml.) and ether (30 ml.).

The material coming off the column in the last five fractions (no. 9–13) showed only very weak carbonyl absorption at 5.88 μ, but did show two definite bands at 2.76 and 2.87 μ. These combined fractions (59 mg.) were reserved for aromatization. Since the materials (64 mg.) from fractions no. 4–8 showed appreciable carbonyl absorption, they were combined for further treatment with methyllithium.

The above-mentioned 64 mg. of material together with several miscellaneous samples of keto ester III weighing 111 mg. were reacetylated with 1 ml. of acetic anhydride in 4 ml. of benzene. Reacetylated material, partially purified by treatment as described above, was obtained as a yellow oil (164 mg.). This was allowed to react with excess ethereal methyllithium in the manner already described. In the chromatography of the product, the following solvents were passed through the alumina column: 2:1 benzene–(30–60°) petroleum ether (25 ml.), 19:1 benzene–ether (30 ml.) and ether (40 ml.). Fractions approximately 7–8 ml. in volume were collected and examined. The crystalline solids obtained from fractions 6–9 were combined with the 59 mg. prepared before and were treated with selenium as described below. This crystalline adduct IV was estimated to contain in the order of 6% of ketonic material. The material, although showing every sign of ready recrystallizability from aqueous methanol, was used without further treatment.

B. **1,7,8,9-Tetramethylphenanthrene (V) from the Selenium Aromatization of Dihydroxy Compound IV.**—A mixture of 122 mg. of dihydroxy compound IV with 250 mg. of powdered selenium in a 15-ml. centrifuge tube was heated at 300° for 7 hours. A smaller test-tube provided with a drip-tip was inserted in the mouth of the centrifuge tube to serve as an air-condenser.

The cooled reaction mixture was extracted with benzene. The selenium remaining was finely ground and further extracted. The combined benzene solutions, after filtration through a layer of diatomaceous earth (Celite), were evaporated in a stream of dry nitrogen. The dark yellow residual oil (41 mg.) was dissolved in a small volume of 30–60° petroleum ether and placed on a 9 × 60 mm. chromatography column containing 3 g. of Fisher alumina. More petroleum ether served as eluting solvent, 7-8-ml. fractions being collected. The combined crystalline products (*ca.* 10 mg.) from fractions 2 and 3 were recrystallized four times from methanol. Fine white needles (0.6 mg.) of 1,7,8,9-tetramethylphenanthrene (V) were obtained in this way with m.p. 107–108°. The 2,4,7-trinitrofluorenone derivative was prepared by treating the warm mother liquors from the second recrystallization with a saturated methanolic solution of 2,4,7-trinitrofluorenone. After two recrystallizations from ethanol–benzene, the deeply colored (orange-red) derivative, which first melted at 159–161°, showed m.p. 164–166°. For preparation of the picrate, 1,7,8,9-tetramethylphenanthrene, m.p. 107–108°, was dissolved in a few drops of methanol, and a saturated methanolic solution of picric acid was added. The intensely orange crystalline picrate, after collection and drying, showed m.p. 139.5-140.5°. The melting points of, and the mixed melting points with, authentic samples were: hydrocarbon, m.p. 108–109.5°, m.m.p. 107–109°; trinitrofluorenone derivative, m.p. 166–168°, m.m.p. 164–166°; picric acid derivative, m.p. 140–141°, m.m.p. 139.5–140°. The values previously observed[7] for synthetic 1,7,8,9-tetramethylphenanthrene and its derivatives are: hydrocarbon, m.p. 109.5–110.5°; trinitrofluorenone derivative, m.p., 166–167°; picrate, m.p. 141.5–142.5°.

The ultraviolet absorption curve was determined using the material in the mother liquors from the final recrystallization of 1,7,8,9-tetramethylphenanthrene. This was diluted with 95% alcohol as necessary. The concentration of the 1,7,8,9-tetramethylphenanthrene was determined by removing solvent completely from a known volume of the solution and weighing the non-volatile hydrocarbon. To the extent that error was introduced by this procedure, the extinctions given for the degradation product in the following table should be regarded as approximate. The absorption curve for the degradation hydrocarbon V and that from authentic synthetic material[7] are identical in appearance. The absorption curve for the degradation hydrocarbon in 95% alcohol was taken at three concentrations: *viz.*, 1.37 × 10^{-5} *M* from 210–280 mμ, 3.4 × 10^{-5} *M* from 280–330 mμ, and 3.4 × 10^{-4} *M* from 330–360 mμ. The absorption curve for the synthetic hydrocarbon was taken[7] in 95% alcohol at concentrations of 1 × 10^{-5} *M* (215–270 mμ), 2 × 10^{-5} *M* (270–320 mμ) and 7.8 × 10^{-4} *M* (320–365 mμ).

ULTRAVIOLET ABSORPTION MAXIMA, Mμ, AND LOG ϵ VALUES FOR 1,7,8,9-TETRAMETHYLPHENANTHRENE, SH = SHOULDER

Degradation material		Synthetic material	
216	(4.53)	217.3	(4.61)
sh230	(4.26)	sh228	(4.33)
sh258	(4.69)	sh257	(4.74)
263.5	(4.73)	264	(4.81)
sh285	(4.04)	sh286	(4.16)
298	(4.01)	298	(4.15)
310	(4.01)	311	(4.18)
sh335	(2.56)	sh336	(2.11)
342	(2.70)	342	(2.55)
350.5	(2.44)	351	(1.55)
358	(2.59)	358	(2.36)

Diketocassenic Acid (VI).—Cassaic acid was oxidized to diketocassenic acid with chromic anhydride essentially according to a published procedure.[9] The crystalline product as obtained directly from the reaction mixture showed m.p. 236–238° (72% yield). Several recrystallizations from aqueous acetone gave diketocassenic acid (VI), m.p. 238–239°.

Anal. Calcd. for $C_{20}H_{28}O_4$: C, 72.26; H, 8.49. Found: C, 72.28; H, 8.41.

The optical rotation was $[\alpha]^{25}D$ −161 ± 1° (0.15% in absolute alcohol); absorption maxima were evident at 218 mμ (log ϵ 4.31) in 3.16 × 10^{-5} *M* absolute ethanolic solution, and at 3.05, 5.81, 5.88 and 6.05 μ in a mineral oil mull. The constants reported before are m.p. 238–239° and $[\alpha]^{20}D$ −164.5° (1% in 95% alcohol).[9]

The Zimmerman test[12] for the presence of a ketone group in the 2-position was made by adding 0.5 mg. of compound to 1 ml. of 2 *N* ethanolic potassium hydroxide and 1 ml. of 1% *m*-dinitrobenzene in absolute alcohol. The test solution with diketocassenic acid (VI) immediately acquired a violet color. The test solution with cassaic acid (I) remained colorless, as did a control solution.

Decarboxylation of Diketocassenic Acid (VI).—Decarboxylation was effected by heating diketocassenic acid (300 mg.) under an atmosphere of nitrogen in a bath at 255 ± 4° for 45 minutes. A solution of the product in approximately 3 ml. of 1:1 benzene–(30–60°) petroleum ether was passed through a chromatography tube (8 mm. i.d.) containing 2 g.

of alumina. More of the same solvent was used for elution. Complete removal of solvent from the first 60 ml. of eluate gave 209 mg. (81%) of crystalline decarboxylation product VII, m.p. 105–108°. Recrystallization from 30–60° petroleum ether brought the melting point to 111.5–113°.

Anal. Calcd. for $C_{15}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 78.88; H, 99.5.

The decarboxylated compound VII showed $[\alpha]^{25}D -60 \pm 2°$ (0.15% in absolute alcohol). A 3.5×10^{-5} *M* solution in alcohol showed no maxima between 210–320 mμ. A mineral oil mull had infrared absorption maxima at 3.24(w), 5.87, 6.04 and 11.05 μ.

Active hydrogen determinations with lithium aluminum hydride at room temperature showed 0.00%, with methyl Grignard reagent in anisole either at 0° or at 100°, 0.19% and with methyl Grignard reagent in toluene 0.0% at 0° and 0.05% at 100°. The value calculated for one active H is 0.35%.

1,2,7,8-Tetramethylphenanthrene (X) from Diketone VII. A. Reaction of Methylmagnesium Iodide with the Diketone.—Grignard reagent was prepared from 136 mg. (5.62 millimoles) of magnesium and 0.35 ml. (5.6 millimoles) of methyl iodide with 15 ml. of ether. The reaction was carried out in a 50-ml. three-necked flask fitted with condenser, drying tube, and magnetic stirrer—all scrupulously dried. An atmosphere of nitrogen was maintained over the reaction. A solution of 108 mg. (0.375 millimole) of decarboxylated diketocassenic acid (VII), m.p. 107–109°, in *ca.* 10 ml. of ether that had been distilled from lithium aluminum hydride was added to the stirred Grignard mixture. After 4 hours, 20 ml. of benzene was distilled from lithium aluminum hydride directly into the reaction mixture, which was then refluxed gently and stirred for 19 hours.

A cold saturated solution of ammonium chloride in water was added to the cooled mixture until no solid remained. The upper layer was separated, and the aqueous layer was extracted with ether. The combined organic solutions were washed first with water and then with saturated aqueous sodium chloride solution. The solution, after drying with sodium sulfate, was distilled to remove all solvent. The yellow, oily residue (118 mg.) in 5 ml. of 30–60° petroleum ether was placed on a chromatography column (8 mm. diameter) containing 2 g. of alumina (Fisher). The following solvents were passed through the column successively, with 7–8-ml. fractions being collected and examined: 30–60° petroleum ether (30 ml.), 1:19 benzene–petroleum ether (30 ml.), 1:9 benzene–petroleum ether (30 ml.), 1:1 benzene–petroleum ether (68 ml.), benzene (45 ml.), ether (35 ml.) and methanol (15 ml.). A total of 116 mg. of product was recovered. Although many of the crystalline fractions showed strong hydroxyl absorption at 2.87 μ as well as carbonyl absorption at 5.88 μ, no sharp melting product could be obtained, even after recrystallizations or repeated chromatography.

B. Treatment of the Grignard Adduct VIII with Lithium Aluminum Hydride.—A dry ether solution of 54 mg. of the adduct VIII was magnetically stirred at room temperature for 20 hours with a suspension of 0.2 g. of lithium aluminum hydride in 10 ml. of dry ether. The mixture then was boiled for 2 hours.

The cooled reaction mixture was carefully treated with a cold saturated aqueous solution of sodium potassium tartrate. The aqueous layer was extracted twice with ether, and the combined ether solutions, after further washing with tartrate solution, water and saturated sodium chloride solution, were dried with sodium sulfate. Removal of all solvent on the steam-bath left 54 mg. of a yellow residue, which in the form of a film showed intense absorption at 2.90 μ but no absorption in the carbonyl region. Attempts at purifying dihydroxy compound IX by crystallization gave various solid and crystalline fractions none of which, however, melted sharply.

C. Selenium Aromatization of Dihydroxy Compound IX.—Diketone VII was treated with methylmagnesium iodide, and the reaction product was processed as described in part A up to the chromatography step. At this point the partially purified hydroxy ketone VIII was carried through the lithium aluminum hydride reduction, and the resulting dihydroxy product IX treated as follows.

A mixture of 262 mg. of dry glassy dihydroxy derivative IX and 500 mg. of black powdered selenium in a 15-ml. centrifuge tube was held in a bath at 315–330° for 8 hours. An 8-mm. test-tube inserted in the mouth of the centrifuge tube served as an air condenser.

The mixture was extracted with several portions of benzene, the residual solids were powdered and then further extracted with benzene. Evaporation of the combined benzene solutions left a black tar, which was dissolved in 20 ml. of warm methanol. Addition of 3 ml. of a saturated methanolic solution of 1,3,5-trinitrobenzene followed by cooling at 0° resulted in the deposition of deep orange crystals, which were collected. More of this material was obtained by adding another 1.5-ml. portion of trinitrobenzene solution to the concentrated filtrate from the first crop.

The combined, desiccated trinitrobenzene derivative (65 mg.) was dissolved in a small volume of carbon tetrachloride, and the solution was passed through a 1 × 6.5 cm. column of Fisher alumina (4 g.). This was followed with more carbon tetrachloride. The first 16 ml. of eluate was taken to dryness, and the faintly yellow residue of 1,2,7,8-tetramethylphenanthrene (X) was crystallized from 4 ml. of methanol to give 11.4 mg., m.p. 150–155°. Four recrystallizations from methanol gave 1.6 mg. of the phenanthrene, m.p. 168.5–169.5° with sintering at 165°. In another aromatization experiment, lustrous plates, m.p. 169–710° (sinter 165°), were obtained.

A sample of synthetic 1,2,7,8-tetramethylphenanthrene,[10] m.p. 170–170.4°, after recrystallization from methanol melted at 170–171°. The mixture melting point of degradation and authentic hydrocarbons was 169–170.5°.

The degradation 1,2,7,8-tetramethylphenanthrene (0.7 mg.) in 0.2 ml. of absolute ethanol was treated with 0.2 ml. of saturated methanolic trinitrobenzene solution. The precipitated trinitrobenzene derivative was recrystallized from ethanol–chloroform to a melting point of 206–207° (sinter at 199°). The trinitrobenzene derivative of synthetic 1,2,7,8-tetramethylphenanthrene, prepared in the same way, showed m.p. 205–207° (sinter 201°). A mixture of the two samples showed m.p. 205–207° (sinter 203°).

A picrate derivative was prepared from degradation 1,2,7,8-tetramethylphenanthrene, m.p. 169–170°, by mixing a saturated methanolic solution of picric acid with a methanolic solution of the hydrocarbon. The deep orange crystals showed m.p. 184–185° (soften at 181°). The picrate prepared from synthetic 1,2,7,8-tetramethylphenanthrene melted at 184–185°. The melting point of a mixture of synthetic and degradation picrate was 184–185°.

Previously reported melting points for 1,2,7,8-tetramethylphenanthrene (X) and its derivatives are, for synthetic material[10]: hydrocarbon, m.p. 170–170.5°; trinitrobenzene derivative, m.p. 209–210°; picrate, m.p. 180–182°, and for degradation material derived from trametenolic acid[18]: hydrocarbon, m.p. 158–159°; trinitrobenzene derivative, 205–206°; picrate, 184–185°.

The infrared absorption spectra of the two samples of hydrocarbon, both as mulls with mineral oil, were identical. The ultraviolet absorption spectra of the two hydrocarbons were identical in general appearance and in the positions of the maxima, which are tabulated below. Insofar as the extinctions reflect possible error in our concentration determinations, no claim for high accuracy in the extinction values can be made.

ULTRAVIOLET ABSORPTION MAXIMA, Mμ, AND LOG ε VALUES FOR 1,2,7,8-TETRAMETHYLPHENANTHRENE (X), SH = SHOULDER

Degradation material	Synthetic material	Synthetic material previously reported[19]
216 (4.44)	216 (4.54)	... ...
256 (4.56)	256 (4.70)	256 (4.75)
263 (4.66)	263 (4.81)	264 (4.82)
285 (4.01)	285 (4.10)	285 (4.19)
295 (3.93)	295 (4.02)	295 (4.15)
308 (4.03)	308 (4.13)	307 (4.22)
326 (2.32)	326 (2.42)	325 (2.61)
... ...	... ...	sh331 (2.42)
341 (2.39)	341 (2.53)	340 (2.68)
358 (2.07)	358 (2.19)	356 (2.32)

(18) W. Gruber and G. Proske, *Monatsh.*, **82**, 255 (1951).

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

The ultraviolet absorption curves for the synthetic and degradation hydrocarbon were determined with absolute alcohol solutions using concentrations of 2.4×10^{-5} M in the 210–315 mμ region and 3.4×10^{-4} M in the 315–360 mμ region. The earlier ultraviolet absorption curve was determined using petroleum ether solutions.[19]

BOSTON, MASS.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

The Cholegenins. II. Structure of Cholegenin, Isocholegenin and Dihydrocholegenin

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It is shown that cholegenin is a 22,25-epoxy-5β-furostane-3α,26-diol (I). Isocholegenin is formulated as spirostane-3α,25-diol (VI). Sodium metaperiodate oxidative cleavage of dihydrocholegenin (II) to a 16,22-epoxynorcoprostan-3α-ol-25-one (III) establishes the structure of II as 16,22-epoxycoprostane-3α,25,26-triol. The partial synthesis of II and III is described.

It was shown in the preceding paper[1] that the catalytic reduction of cholegenin and isocholegenin with platinum oxide in glacial acetic acid at 25° gave only one dihydrocholegenin which upon acetylation yielded a monohydroxy diacetate. The unacylable hydroxyl group was formed by the reductive opening of an epoxide ring of cholegenin and was tentatively placed at C-25 of dihydrocholegenin. It had been established previously[2] that the primary hydroxyl group of cholegenin was in the side chain. If the primary and tertiary hydroxyl groups of dihydrocholegenin are at C-26 and C-25, respectively, then dihydrocholegenin possesses a 1,2-glycol linkage. Oxidation of dihydrocholegenin with sodium metaperiodate in aqueous dioxane gave a precipitate of sodium iodate within five minutes. From the reaction mixture a compound was obtained, in quantitative yield, whose infrared spectrum exhibited strong hydroxyl absorption at 3571 cm.$^{-1}$ (unassociated) and a very strong carbonyl band at 1716 cm.$^{-1}$. Elemental analysis agreed with the empirical formula $C_{26}H_{42}O_3$, showing the loss of one carbon atom. Since the structural formula of cholegenin up to C-22 appears to be well established[2] the oxidation product III must be a 16,22-epoxynorcoprostan-3α-ol-25-one, and dihydrocholegenin a 16,22-epoxycoprostane-3α,25,26-triol (II).

In order to confirm further the structure of II and III, these compounds were synthesized from 16,22-epoxycoprost-25-en-3α-ol (IVa).[1] Acetylation of IVa and subsequent hydroxylation of the acetate IVb with osmium tetroxide in ether and a two-step hydrolysis (aqueous ethanolic sodium sulfite, 2% potassium hydroxide solution) gave II in a yield of 75%. Hydroxylation of IVa with Woodward and Brutcher's[3] more convenient reagent (iodine, silver acetate and wet acetic acid) gave II in a yield of 68%. Compound II obtained from I and from IV proved to be identical. Also the infrared spectra of the diacetates in chloroform and carbon disulfide solution were indistinguishable. In either procedure only one of the C-25 epimers of II was formed. Oxidation of IVb[1] and subsequent hydrolysis of the 3α-acetoxy group gave the 16,22-epoxynorcoprostan-3α-ol-25-one III in a yield of 85%. The compound III samples obtained from II and IVa were identical.

We obtained by oxidation of cholegenin in acetone with chromic acid in dilute sulfuric acid a keto-acid VIII (containing 27 carbons) identical with that of Mazur and Spring[2] formed by oxidizing cholegenin with chromic acid in 80% acetic acid. An identical keto-acid also was formed when isocholegenin was oxidized under our conditions. When this keto-acid (derived from cholegenin or isocholegenin) was reduced with lithium aluminum hydride, cholegenin was obtained in nearly quantitative yields, when no acid was used in the working up process.[4] We found, on the other hand, that cholegenin is very readily and nearly quantitatively isomerized to isocholegenin with dilute ethanolic hydrochloric acid at room temperature in 30 minutes, *i.e.*, under considerably milder conditions than previously had been employed for cholegenin[5] or for C-25 epimerizations.[6]

Establishment of the structure of II together with the finding of Mazur and Spring[2] that drastic oxidation of cholegenin diacetate leads to a 3α-acetoxy-16β-hydroxy bisnorcholanic lactone (VII) allows placement of the oxygen bridge of ring F at C-22 and C-25 and assignment of the structure of 22,25-epoxy-5β-furostane-3α,26-diol for cholegenin (I). According to Callow and Massy-Beresford[7] the C-22 oxygen of the F-ring of the steroidal sapogenins is α-oriented. Since ketone III can be prepared from cholegenin (*via* II) and from sarsasapogenin or smilagenin (*via* IV) it may be concluded that the C-22 oxygen of cholegenin is α-oriented.

Having assigned structure I to cholegenin, we

(1) Previous paper, Malcolm J. Thompson, Irving Scheer and Erich Mosettig, THIS JOURNAL, **81**, 5225 (1959).

(2) Y. Mazur and F. S. Spring, *J. Chem. Soc.*, 1223 (1954).

(3) R. B. Woodward and F. V. Brutcher, Jr., THIS JOURNAL, **80**, 209 (1958).

(4) A similar reduction had been carried out by Mazur and Spring[2] who used acetic acid for the decomposition of the reaction mixture. Because of the ease of isomerization of cholegenin to isocholegenin under acidic conditions we employed alkaline decomposition. We assured ourselves in separate experiments that neither cholegenin nor isocholegenin are affected by alkali or lithium aluminum hydride under conditions prevailing during the reduction or isolation.

(5) N. J. Anita, Y. Mazur, R. R. Wilson and F. S. Spring, *J. Chem. Soc.*, 1218 (1954).

(6) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 846 (1939).

(7) R. K. Callow and P. N. Massy-Beresford, *J. Chem. Soc.*, 4482 (1957).