aqueous bicarbonate solution, dried, and evaporated. The residual light brown oil (0.14 g.) crystallized from petroleum ether to give 0.09 g. (27%) of *ar*-2-tetralol, m.p. and mixed m.p. 59°.[11]

Acidification of the bicarbonate wash gave 0.075 g. (17%) of the carboxytetralol (V. R = H), m.p. and mixed m.p. 200–202°.

1,3,4-Tricarbethoxy-ar-2-tetralol (VII. R = C_2H_5). As reported previously,[10] this was made by the condensation of ethyl cyclohexane-1-one-2-glyoxalate and diethylacetone dicarboxylate in the presence of anhydrous sodium ethoxide. The product (crude 75%), a pale yellow oil, gave a violet coloration with ferric chloride, and was used for the following step without further purification.

Hydrolysis and decarboxylation of the triester (VI. R = C_2H_5). A. *With concd. hydrochloric acid alone.* The triester (0.5 g.) was heated to reflux in 5 cc. of concd., hydrochloric acid for 2 hr., and the starting ester (0.45 g.) was recovered unchanged.

B. *With concd. hydrochloric acid plus acetic acid.* A solution of 0.45 g. of the triester in 10 cc. of concd. hydrochloric acid and 7 cc. of glacial acetic acid was heated to reflux on an oil bath (160–170°) for 4 hr. The mixture was concentrated under reduced pressure, and the residual oil was mixed with water, and extracted with ether. The ethereal extracts were washed with aqueous bicarbonate; acidification of the bicarbonate wash furnished 0.16 g. (36%) of the tetralol-monoester diacid as a crystalline solid, m.p. 151–153° dec. Recrystallization from ethyl acetate–hexane formed colorless prisms m.p. 171–173° dec.; $\lambda_{max}^{C_2H_5OH}$ 220 mμ (ε 23,878), 275 mμ (ε 3858), 350 mμ (ε 5629). It showed a violet coloration with ferric chloride.

Anal. Calcd. for $C_{15}H_{16}O_7$: C, 58.44; H, 5.23. Found: C, 58.43; H, 5.34.

The ethereal layer, removed from the bicarbonate wash, gave 0.14 g. (32%) of the starting ester.

C. *With alkali.* A solution of 6.9 g. of the triester in 80 cc. of 10% methanolic potassium hydroxide was heated to reflux for 3 hr. Worked up as usual, the bicarbonate-soluble fractions gave a brown mass, which crystallized from ethyl acetate: VII (R = H), 4.86 g., 92%. Further recrystallization from ethyl acetate–hexane formed colorless prisms, m.p. 228–230° dec.; $\lambda_{max}^{C_2H_5OH}$ 215 mμ (ε 25,257), 324.5 mμ (ε 4835), 353 mμ (ε 3102). It gave a violet coloration with ferric chloride.

Anal. Calcd. for $C_{13}H_{12}O_7$: C, 55.72; H, 4.33. Found: C, 55.79; H, 4.44.

D. *Selective didecarboxylation of the above triacid* (VII. R = H). This was carried out by an application of the previous method[10] used for the direct conversion of the triester into the monoacid. A solution of 1.0 g. of the above triacid in 70 cc. of concd. hydrochloric acid and 30 cc. of glacial acetic acid was violently refluxed at 180–190° (bath temperature) for 25 hr. The reaction solution was allowed to stand overnight at room temperature, giving 0.60 g. (88%) of the desired monoacid (V. R = H), m.p. 197–200°. The mother liquors afforded an additional 0.06 g. (total 0.66 g., 97%). Recrystallization from ethyl acetate–hexane formed colorless prisms, m.p. 201–203° [15]; $\lambda_{max}^{C_2H_5OH}$ 215 mμ (ε 11,535), 306 mμ (ε 2611); the infrared spectrum exhibited a strong band at 867 cm.$^{-1}$, due to isolated hydrogens on a benzene ring.[2b] An analytical sample was dried at 120° and 3 mm.

Anal. Calcd. for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29. Found: C, 68.68; H, 6.44.

An ethyl ester (V. R = C_2H_5), was made quantitatively with ethanol–sulfuric acid as colorless needles, m.p. 124–126° (from dilute methanol); $\lambda_{max}^{C_2H_5OH}$ 213 mμ (ε 18,060), 306 mμ (ε 3014); the infrared spectrum exhibited two strong bands at 878 and 858 cm.$^{-1}$, due to isolated hydrogens on a benzene ring.[2b] An analytical sample was dried at 80° and 3 mm.

Anal. Calcd. for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 70.91; H, 7.20.

ar-2-Tetralol. This was prepared by catalytic hydrogenation of 2-naphthol as reported previously[11]; m.p. 59° (from petroleum ether). A *p-nitrobenzoate* was made with benzoyl chloride; m.p. 110–112° (from methanol).

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(15) Prelog *et al.*[10] have reported briefly that, on heating with hydrochloric acid in a sealed tube at 180°, the triester (VII. R = C_2H_5) gave an unspecified yield of the monoacid (V. R = H), m.p. 203°.

Stereospecific Oxidation of a Methylthio Steroid to the Sulfoxide by *Calonectria decora*

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Strains of the culture *Calonectria decora* have been reported to modify steroids by processes involving 1,2-dehydrogenation[1] and hydroxylation at C-12 and C-15.[2] We have now found that fermentation of 17β-acetoxy-7α-methylthioandrost-4-en-3-one (I) with *Calonectria decora* (CBS) resulted in hydrolysis of the acetate and oxidation of the methylthio group to yield 17β-hydroxy-7α-methylsulfinylandrost-4-en-3-one (II).

The structure of II was tentatively assigned on the basis of its elemental analysis, infrared spectrum, and conversion to 17β-hydroxyandrosta-4,6-dien-3-one (III). However, comparison of the acetate IV derived from acetylation of II with a synthetic sample of V[3] prepared by the monoperphthalic acid oxidation of I showed differences in melting point, rotation, and papergram mobility.

Confirmation of the structure assigned to II was provided by oxidation of IV with monoperphthalic acid to yield 17β-acetoxy-7α-methylsulfonylandrost-4-en-3-one (VI), which was identical with a sample obtained by peracid oxidation of I.

(1) E. Vischer, Ch. Meystre, and A. Wettstein, *Helv. Chim. Acta*, **38**, 835 (1955).

(2) A. Schubert, G. Langbein, and R. Siebert, *Chem. Ber.*, **90**, 2576 (1957). Schubert and Siebert, *ibid.*, **91**, 1856 (1958), describe the product from progesterone as 12β,15α-dihydroxy-progesterone. A product obtained from the fermentation of 11β-hydroxyprogesterone with *C. decora* was characterized as the 11β,15α-dihydroxylated derivative by Schubert, Siebert, and Koppe, *Angew. Chem.*, **70**, 742 (1958).

(3) R. E. Schaub and M. J. Weiss, *J. Org. Chem.*, submitted for publication.

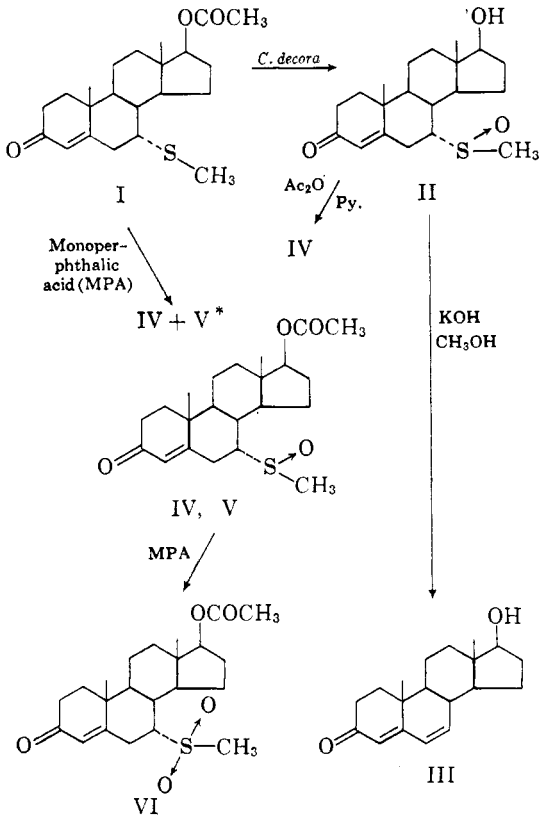

Figure 1

* Compounds IV and V are stereoisomeric about the sulfur atom.

The same sulfone VI was also prepared by similar oxidation of V.[3] Since IV and V were demonstrably dissimilar, and yet yielded the same sulfone, it was concluded that they differed only in the configuration about the sulfur atom. This interpretation was strengthened when it was learned that both IV and V may be obtained by peracid oxidation of I.[3]

Although numerous examples of the isolation of asymmetric sulfoxides from biological systems exist,[4] and it is known that thioethers may be metabolized in animals to sulfoxides,[5-10] the current report represents the first stereospecific oxidation of sulfur in the steroid series by a microorganism.[11]

EXPERIMENTAL[12]

17β-Hydroxy-7α-methylsulfinylandrost-4-en-3-one (II). *Calonectria decora* (CBS) was maintained on oatmeal agar slants. Inoculum for the fermentation was prepared by suspending the microbial growth from three slants in sterile deionized water, and with these suspensions seeding four 500-ml. Erlenmeyer flasks containing 100 ml. each of a sterile medium consisting of 5% glucose, 0.5% corn steep liquor, 0.05% ferrous sulfate heptahydrate, 0.05% magnesium sulfate heptahydrate, 0.05% potassium chloride, 0.1% dipotassium phosphate, and 0.2% sodium nitrate at *p*H 7.0. The mycelium resulting from the incubation of these flasks for 72 hr. on a reciprocating shaker at 28°, was used to inoculate (a 5% inoculum by volume) fifty 500-ml. Erlenmeyer flasks, each containing 100 ml. of the aforementioned medium. After 24-hr. incubation under the above conditions, 20 mg. of I, dissolved in 1.0 ml. methanol, was added to each flask. After incubation for an additional 52 hr., the steroid was recovered from the filtrate by extraction with ethyl acetate (three 1-vol. portions). The concentrated extract was purified by partition chromatography on a 250-g. Celite[13] column moistened with the lower phase of a water–dioxane–cyclohexane solvent system in the volume ratio 1:5:3. After development with 10 vol. of upper phase from the above system, the steroidal product was obtained. Concentration of the appropriate fraction and crystallization yielded 299 mg. of II, m.p. 192.5–193.5°, dec. Similar workup of the mycelial extract yielded an additional 19 mg., m.p. 190–190.5° dec. Recrystallization from acetone gave 271 mg. of II, m.p. 189.5–189.8° dec., $[\alpha]^{23}D$ +11° (0.49% in methanol), $\lambda_{max}^{CH_3OH}$ 246 mμ, ε 11,100.

Anal. Calcd. for $C_{20}H_{30}O_3S$ (350.51): C, 68.53; H, 8.63. Found: C, 68.25; H, 8.72.

17β-Hydroxyandrosta-4,6-dien-3-one (III). A solution of 50 mg. of II in 24 ml. of methanol was treated with 0.5 ml. of a 9% aqueous potassium hydroxide solution. After 70 min. at room temperature the reaction mixture was evaporated *in vacuo*, and the residue was crystallized twice from aqueous methanol to give 23 mg. of III, m.p. 200.5–201.5°, $\lambda_{max}^{CH_3OH}$ 284.5 mμ, ε 26,800, $[\alpha]^{26}D$ +103° (chloroform); Djerassi[14] reports m.p. 204–205°, $[\alpha]^{25}D$ +76.6 (chloroform). Another sample prepared by similar treatment of I had $[\alpha]^{25}D$ +90° (chloroform), m.p. 202.5–203°, $\lambda_{max}^{CH_3OH}$ 284 mμ, ε 26,300.

17β-Acetoxy-7α-methylsulfinylandrost-4-en-3-one (IV). Acetylation of 140 mg. of II in 9 ml. of pyridine and 1 ml. of acetic anhydride at room temperature for 24 hr. yielded, after isolation and three crystallizations from acetone–petroleum ether, 84 mg. of IV, m.p. 172° dec., $[\alpha]^{25}D$ +16.6° (chloroform). This product was identical with a synthetic preparation of IV obtained from Weiss and Schaub.[3] No melting point depression was observed on mixture, and no separation could be achieved by paper chromatography. Comparison with V, also provided by the above authors, showed considerable differences in papergram mobility and in melting points. On paper chromatograms IV moved with R_f = 0.56, while the R_f of V was 0.42.[15]

17β-Acetoxy-7α-methylsulfonylandrost-4-en-3-one (VI). A solution of 50 mg. of IV in 5 ml. of dichloromethane was treated with a slight excess of an ethereal solution of monoperphthalic acid at room temperature. After 1.5 hr. the solution was decanted from the phthalic acid, extracted with

(4) F. Challenger, "Aspects of the Organic Chemistry of Sulphur," Academic Press, New York, 1959, Chap. II.

(5) N. T. Clare, *Australian Vet. J.*, **23**, 340 (1940).

(6) R. B. March, R. L. Metcalf, T. R. Fukuto, and M. G. Maxon, *J. Econ. Entomol.*, **48**, 355 (1955).

(7) N. P. Salzman and B. B. Brodie, *J. Pharmacol., Exp. Therap.*, **118**, 46 (1956).

(8) J. J. Burns, T. F. Yü, A. Ritterband, J. M. Perel, A. B. Gutman, and B. B. Brodie, *ibid.*, **119**, 418 (1957).

(9) S. S. Walkenstein and J. Seifter, *ibid.*, **125**, 283 (1959).

(10) J. R. Gillette and J. J. Kamm, *ibid.*, **130**, 262 (1960).

(11) Stereospecific oxidation of biotin to biotin *l*-sulfoxide by *Aspergillus niger* has been reported by L. D. Wright, E. L. Cresson, J. Valiant, D. E. Wolf, and K. Folkers, *J. Am. Chem. Soc.*, **76**, 4163 (1954).

(12) All melting points are corrected. Melting points of the 7-thiosteroids were extremely susceptible to changes in the rate of heating.

(13) Celite is the trademark of a diatomaceous siliac product prepared by Johns Manville Co.

(14) C. Djerassi, G. Rosenkranz, J. Romo, St. Kaufmann, and J. Pataki, *J. Am. Chem. Soc.*, **72**, 4534 (1950).

(15) The solvent system consisted of benzene, acetic acid, petroleum ether (b.p. 90–100°), water in the volume ratio 13:16:7:4.

sodium bicarbonate solution, washed with water, and dried over magnesium sulfate. The filtrate was concentrated, diluted with ethyl acetate, and the product (VI), 45.5 mg., m.p. 148–151° dec., was crystallized from ethyl acetate–petroleum ether. The product was further purified by partition chromatography on a 20-g. Celite[18] diatomaceous earth column using a solvent system composed of water, methanol, dioxane, and cyclohexane in the volume ratio 2:2:6:10. This system gave an apparent separation into two fractions which were shown to be identical. The main fraction provided 17.5 mg. of VI, m.p. 154–155° dec., $[\alpha]^{25}D$ +21.7° (chloroform). This product and a sample of VI prepared from 7α-methylthiotestosterone acetate (I) with monoperphthalic acid as described above, were identical by infrared absorption and paper chromatographic data. A mixture melting point showed no depression.

Anal. Calcd. for $C_{22}H_{32}O_5S$ (408.54): C, 64.67; H, 7.90. Found: C, 64.24; H, 8.12.

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Chemistry of *Aegiceras majus* Gaertn. IIb. Isolation of 28-Norolean-12,17-dien-3β-ol

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The isolation of genin-A and isorhamnetin from the bark of *Aegiceras majus* Gaertn (Syn. *A. corniculatum* Blanco) was reported earlier.[1] Herein is described the isolation of the first naturally occurring C_{29} triterpene alcohol[2] and its identification as 28-norolean-12,17-dien-3β-ol[3] (Ia) (norechinocystadienol[4]) by direct comparison[5] with an authentic specimen.

The dienol (Ia) was isolated by acid hydrolysis of the glycosides of the bark followed by chromatography of the neutral fraction and was purified *via* its benzoate derivative. It gave a pink color in the Liebermann-Burchard reaction and its analysis conformed to a molecular formula $C_{30}H_{48}O$ (± CH_2). It formed an acetate (Ib) and a benzoate and the nature of the oxygen function as hydroxyl was confirmed by their infrared spectra (*vide* experimental). Oxidation of the dienol with chromium trioxide–pyridine reagent[6] led to the dienone (Ic) which formed an oxime, gave a positive Zimmermann reaction[7] for 3-keto group and on sodium borohydride reduction furnished the dienol again.

The dienol developed a light brown color with tetranitromethane for unsaturation and had absorption maxima in the ultraviolet at 237 and 244 mμ for a conjugated heteroannular diene. Hydrogenation of the dienyl acetate (Ib) over platinum in acetic acid yielded a dihydro derivative which was identified as 28-norolean-17-en-3β-yl acetate[8] (IIb) by direct comparison[9] of the corresponding ketone (IIc) with an authentic sample.

EXPERIMENTAL[10]

Isolation of the dienol. Air-dried powdered bark (1.2 kg.) of *A. majus*[11] Gaertn was defatted with light petroleum and then extracted with 95% and 70% ethanol under reflux and the solvent removed. The residue was freed from non-glycosidic material by two precipitations of its solution in minimum amounts of 95% ethanol with large volumes of ether. The ether-insoluble glycosidic portion was heated under reflux for 4 hr. with aqueous ethanol (1:1, 1 l.) and concentrated hydrochloric acid (200 cc.). The aglycones were collected, washed, dried, and extracted with benzene in a Soxhlet apparatus for 30 hr. The residue from the benzene extract after removal of genin-A as described before[1] was chromatographed in benzene solution on alumina (300 g.). Elution with light petroleum-benzene (3:2) and subsequent crystallization of the residue from methanol, yielded the crude dienol (0.6 g.) as colorless needles, m.p. 170–178°.

28-Norolean-12,17-dien-3β-yl benzoate. The crude dienol (600 mg.) was benzoylated with benzoyl chloride (2 cc.) and pyridine (5 cc.) for 2 hr. at 100°. The reaction product was poured into water and isolated by means of ether. Two crystallizations of the residue (left on removal of ether) from benzene-ethanol yielded the dienyl benzoate as prisms, m.p. 229–231°, $[\alpha]D$ +81° (*c*, 2.25); lit.,[3] m.p. 227–229°, $[\alpha]D$ +81°; lit.,[4] m.p. 231–233°.

(1) Part I, K. V. Rao and P. K. Bose, *J. Indian Chem. Soc.*, **36**, 358 (1959).

(2) Referred to as aegiceradienol in our brief communication: Part IIa, K. V. Rao and P. K. Bose, *Science & Culture*, **24**, 486 (1959).

(3) D. H. R. Barton and C. J. W. Brooks, *J. Chem. Soc.*, 257 (1951).

(4) C. R. Noller and J. F. Carson, *J. Am. Chem. Soc.*, **63**, 2238 (1941).

(5) We are greatly indebted to Prof. C. R. Noller, Stanford University, Calif., for the mixed m.p. and infrared comparison of the benzoates.

(6) G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953).

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

(8) A. Winterstein and G. Stein, *Z. Physiol. Chem.*, **202**, 222 (1931).

(9) We thank Prof. D. H. R. Barton, F.R.S., Imperial College, London, for the mixed m.p. determination.

(10) All melting points are uncorrected. Ultraviolet absorption spectra were done on a Beckman model DU quartz spectrophotometer in ethanol solution and optical rotations were determined in chloroform at room temperature. Light petroleum refers to fraction, b.p. 60–80°, and alumina used is of Brockmann's (E. Merck) grade. Samples for analysis were dried *in vacuo* over phosphorus pentoxide at 110° for 12 hr.

(11) The plant material was collected for us by the Divisional Forest Officer, 24 Parganas, West Bengal, to whom our thanks are due.