epimer into the acetonide, the 3β,5α,6β-triol was recovered unchanged. Oxidation of the 3β,5α,6β-triol with sodium dichromate in acetic acid gave, after chromatography and in 27% yield, $\Delta^{7,22}$-ergostadiene-3,6-dione-5α-ol,[9] identical with a sample prepared by oxidation of the 3β,5α,6α-triol; m.p. 254–255° dec., α_D +46° Chf (*c* 0.5), λ^{EtOH} 249 mμ (11,750).

Anal. Calcd. for $C_{28}H_{42}O_3$ (426.62): C, 78.82; H, 9.92. Found: C, 78.65; H, 10.24.

Perbenzoic Acid Oxidation of Ergosteryl Acetate (W.E.R.).—An ice-cold chloroform solution of 7.2 g. of perbenzoic acid (1 equiv.) was added during 45 min. to an ice-cold solution of 22 g. of ergosteryl acetate in 500 cc. of chloroform. The solution was let stand at 0–5° for 20 hr. and then washed with aqueous carbonate solution, dried and evaporated below 60° in vacuum to a light yellow solid. The relative intensity of ultraviolet absorption at 231 mμ (benzoate) and at 324 mμ ($\Delta^{5,7,9(11)}$-triene) indicated the presence of about 2 parts of $\Delta^{7,22}$-ergostadiene-3β,5α,6α-triol 3-acetate 6-benzoate to 1 part of dehydroergosteryl acetate, and these two products were separated by chromatography. Dehydroergosteryl acetate (less soluble in acetone–methanol), was identified by analysis, comparison with an authentic sample, and its physical constants: m.p. 144.5–145.5°, α_D +184° Chf (*c* 2.02), λ^{EtOH} 311, 324, 340 mμ (9,600; 10,800; 6,670). $\Delta^{7,22}$-Ergostadiene-3β,5α,-6α-triol 3-acetate 6-benzoate, m.p. 190–191° from acetone–methanol (lit.[8] 186–187°), on hydrolysis with Claisen alkali gave the triol, which crystallized from ethyl acetate as white, rectangular prisms, m.p. 243.5–244.5°, α_D +30° Py (*c* 0.97); lit.[8] m.p. 241–242°, α_D +29° Py. The 3,6-diacetate melted at 180–181°, α_D +42° Chf (*c* 1.23), +10° Py (*c* 1.34); lit.[11] m.p. 181–182°, α_D +41° Chf.

CAMBRIDGE, MASSACHUSETTS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MONTANA STATE UNIVERSITY]

Synthetic Estrogens. II.[1] Derivatives of 2-Phenylphenanthrene and 1-Ethyl-2-benzylnaphthalene

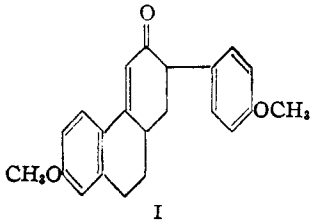
BY RICHARD E. JUDAY

RECEIVED MARCH 9, 1953

Derivatives of 2-phenylphenanthrene have been made using the method of DuFeu, McQuillan and Robinson starting with 6-methoxy-α-tetralone and anisylacetone. The derivatives of 1-ethyl-2-benzylnaphthalene were prepared by alkylating 6-methoxy-α-tetralone followed by reaction with ethylmagnesium bromide. Three of the four compounds tested showed activity at the 1 mg. level.

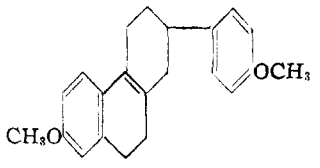
In connection with work on derivatives of 1-phenylphenanthrene,[1] it was desired to compare their biological activity with corresponding 2-substituted isomers and with compounds in which ring C had been opened.

The reaction between 6-methoxy-α-tetralone and 1-dimethylamino-2-(*p*-methoxyphenyl)-3-butanone methiodide using the method of DuFeu, McQuillan and Robinson[2] was not successful. The modification of Shunk and Wilds[3] using the 2-hydroxymethylene derivative of 6-methoxy-α-tetralone was successful, giving a yield of 46% of product, m.p. 187–188.5°. This compound had an ultraviolet absorption spectrum closely resembling that of the 1-substituted isomer[1] with a maximum at 330 mμ, indicating that it was the expected 2-(*p*-methoxyphenyl)-3-keto-7-methoxy-1,2,3,9,10,-10a-hexahydrophenanthrene (I). Reduction of I using the modification of the Wolff–Kishner tech-

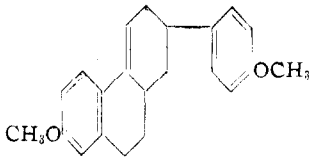


I

nique previously outlined[1] produced a mixture, which was separated with some difficulty by fractional crystallization and chromatographing on alumina, into II, m.p. 148–151°, and III, m.p. 121–123.5°. Consideration of the ultraviolet absorption



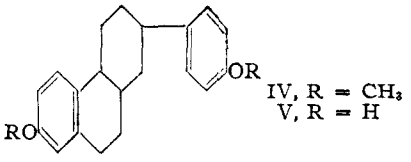
II



III

spectra indicated that II, with a maximum at 272.5 mμ has the double bond in the endo position, while in III, with a maximum at 264.5 mμ, the double bond is located in the exo position.[1,4]

Catalytic hydrogenation of a mixture of II and III produced a single isomer of IV, m.p. 91.5–93°, in good yield. Cleavage of the methoxyl groups of IV with hydrobromic acid produced V, m.p. 194–196°.



Since only the one isomer was obtained from the reduction of II and III, the most likely B:C configuration of IV is *cis*, because reduction of the endo double bond should give rise to that configuration exclusively. If the results found by Linstead and

(1) R. E. Juday, Paper I, THIS JOURNAL, **75**, 3008 (1953).

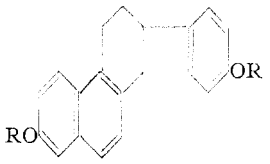
(2) E. C. DuFeu, F. J. McQuillan and R. Robinson, *J. Chem. Soc.*, 53 (1937).

(3) C. H. Shunk and A. L. Wilds, THIS JOURNAL, **71**, 3946 (1949).

(4) J. Heer and K. Miescher, *Helv. Chim. Acta*, **31**, 219 (1948).

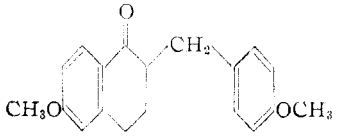
co-workers[1,5abc] are applicable here, the configuration between C_2–C_{10a} should also be *cis*, with a *cis*,*syn*-configuration for the three asymmetric centers. No definite conclusion can be formed on the latter point, although the low melting points of both IV and V compared with other members of this series and the 1-substituted isomers[1] is compatible with this formulation.

Treatment of the mixture of II and III with palladium–charcoal catalyst in cyclohexene solution resulted in disproportionation rather than dehydrogenation as was the case with the 1-substituted isomer.[1] Here, however, the tetrahydro derivative, VI, m.p. 170.5–172.5°, was higher melting and less soluble than the octahydro compound so it was readily isolated in a pure state. Cleavage of the methoxyl groups to form VII was best effected with pyridine hydrochloride.



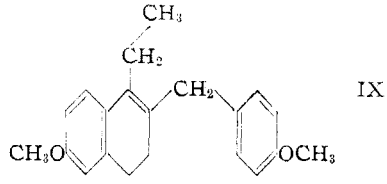
VI, R = CH_3
VII, R = H

Alkylation of sodio-6-methoxy-α-tetralone with *p*-methoxybenzyl chloride produced the ketone VIII, which reacted readily with ethylmagnesium bromide to produce IX, but failed to react either with semicarbazide or dinitrophenylhydrazine.



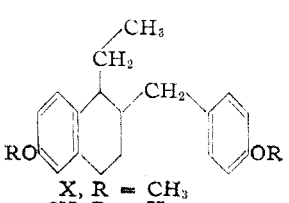
VIII

The absorption maximum of IX, at 270 mμ, is intermediate between values previously found[1,4] for the exo and endo positions of the double bond, but seems to favor the endo position with some of the other isomer probably present. Since the product was an oil no purification beyond distillation was effected.

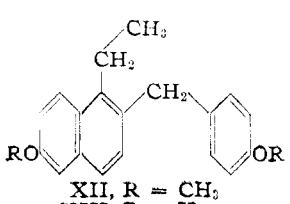


IX

Catalytic hydrogenation of IX produced X, also an oil, whose methoxyl groups were readily cleaved with hydrobromic acid to give XI, m.p. 116–120°. The relatively poor melting point of XI is also an indication that IX was a mixture of isomers which could result in a mixture of *cis*,*trans* isomers on reduction.



X, R = CH_3
XI, R = H



XII, R = CH_3
XIII, R = H

(5) (a) R. P. Linstead and A. L. Walpole, *J. Chem. Soc.*, 842 (1939); (b) R. P. Linstead and A. L. Walpole, *ibid.*, 850 (1939); (c) R. P. Linstead and W. E. Doering, THIS JOURNAL, **64**, 1991 (1942).

Dehydrogenation of X was best achieved by heating with palladium–charcoal catalyst. Sulfur was found to be unsatisfactory. The crude product was purified by picrate formation to give XII, m.p. 71–72°. Cleavage of the methoxyl groups with pyridine hydrochloride gave XIII, m.p. 164–166°.

Results of the bioassays for estrogenic activity are given in Table I.

TABLE I

ESTROGENIC ACTIVITIES

	Compound	Total dose,[a] mg.	No. of animals	% positive response
1	2-(*p*-Hydroxyphenyl)-7-hydroxy-1,2,3,4-tetrahydrophenathrene (VII)	0.5	10	10
		1.0	10	20
2	2-(*p*-Hydroxyphenyl)-7-hydroxy-1,2,3,4,4a,9,10,10a-octahydrophenanthrene (V)	0.5	10	0
		1.0	10	10
3	1-Ethyl-2-(*p*-hydroxybenzyl)-6-hydroxy-1,2,3,4-tetrahydronaphthalene (XI)	0.5	8	37
		1.0	11	36
4	1-Ethyl-2-(*p*-hydroxybenzyl)-6-hydroxynaphthalene (XIII)	0.5	4	0
		1.0	10	20

[a] Two doses injected subcutaneously in sesame oil into rats.

It will be noted that the compounds tested have about the same order of activity as those reported previously.[1] Shifting the *p*-hydroxyphenyl group to the 2-position as in compound VII and opening the C ring as in compound XI had little effect on activity. It will also be noted that compound XI has slightly more activity than the corresponding *p*-hydroxyphenyl substituted analog.[6]

Acknowledgments.—The author is indebted to the Research Corporation and the research committee of Montana State University for funds to support this work and wishes to express his gratitude to Drs. L. Earle Arnow and J. M. Sprague of Sharp and Dohme, Inc., for the estrogenic assays.

Experimental[7]

2-Hydroxymethylene-6-methoxy-α-tetralone.—The condensation between 6-methoxy-α-tetralone and ethyl formate was carried out using a modification of the method of v. Auwers and Wiegand[8] who used sodium as catalyst. A mixture of 10.6 g. of 6-methoxy-α-tetralone, 13.3 g. of ethyl formate, 3.0 g. of sodium hydride (ground under benzene), 50 ml. of dry ether and 100 ml. of dry ether was refluxed with stirring in an argon atmosphere until the vigorous reaction which ensued appeared to be over, and for an additional half hour. The reaction mixture was worked up using the method of Wilds and Shunk[3] and the product obtained on evaporation of the solvent recrystallized from methanol to give 9.8 g. (80%) of slightly tan product, m.p. 65–67°. This product was satisfactory for the next step but could be purified further by decolorizing in benzene, 60° naphtha solution with Norite and recrystallizing in methanol to give an almost white product, m.p. 68–69° (vac.).

Anal. Calcd. for $C_{12}H_{12}O_3$: C, 70.59; H, 5.88. Found: C, 70.88; H, 5.92.

(6) E. C. Dodds, L. Goldberg, W. Lawson and R. Robinson, *Proc. Roy. Soc.* (*London*), **B127**, 140 (1939).

(7) All melting points in this paper are corrected.

(8) K. v. Auwers and C. Wiegand, *J. prakt. Chem.*, [2] **134**, 82 (1932).

1-Anisyl-2-nitropropene.—The modification of the method of Knoevenagel and Walter[9] developed by Cope[10] was followed using morpholine–morpholine acetate as catalyst. The anisaldehyde was refluxed with a 50% excess of nitroethane in benzene–toluene solution for 17.5 hours. The mixture was then distilled until 500 ml. of distillate was collected. The residue was cooled, washed with water and dried over magnesium sulfate. The mixture was filtered and the filtrate evaporated and distilled *in vacuo*. The distillate was then recrystallized from methanol to give 150.6 g. (62%) of product, m.p. 44–46° (48°).[9]

Anisylacetone.—The 1-anisyl-2-nitropropene was reduced using the method of Hass, Susie and Heider[11] except that reduced iron was used instead of granular iron. A yield of 68% of product distilling at 134–136° (10 mm.), $n^{20}D$ 1.5251 (1.5253),[11a] was obtained. The semicarbazone, m.p. 169–170.5°, was submitted for analysis.

Anal. Calcd. for $C_{11}H_{15}O_2N_3$: N, 19.00. Found: N, 19.11.

1-Dimethylamino-2-(*p*-methoxyphenyl)-3-butanone Methiodide.—A mixture of 24.6 g. of anisylacetone, 8.0 g. of paraformaldehyde, 12.5 g. of dimethylamine hydrochloride and 17 ml. of absolute ethanol was refluxed and stirred for four hours in a 300-ml. 3-necked flask. The bulk of the alcohol was removed by vacuum distillation and the residue diluted with water and filtered through Filter-cel. The filtrate was made alkaline with 100 ml. of concentrated ammonia and extracted twice with ether. The combined ether extracts were washed with 10% brine, saturated brine and dried over magnesium sulfate. The mixture was filtered and the filtrate treated with 20 ml. of methyl iodide and allowed to stand overnight at room temperature. The product was filtered, washed with ether and dried. The product was suspended in 60 ml. of methanol, boiled and filtered. The filtrate was diluted to 600 ml. with ether, chilled and filtered and the product dried *in vacuo* over phosphorus pentoxide. The yield was 23.5 g. (36%) of product which decomposed on heating between 170–177°.

Anal. Calcd. for $C_{14}H_{22}O_2NI$: I, 34.98. Found: I, 35.23.

Proof of Structure.—The water solution containing the Mannich base from 12.3 g. of anisylacetone was made alkaline with 20% potassium hydroxide and extracted twice with ether. The ether extracts were washed with 10% brine, saturated brine and dried over magnesium sulfate. The solution was evaporated *in vacuo*, the residue taken up in 50 ml. of ethanol and stirred in a hydrogen atmosphere for two days over 0.8 g. of 30% palladium–charcoal catalyst. Based on the yield of product, a little over one mole of hydrogen was absorbed. The reaction mixture was filtered and the filtrate concentrated and distilled *in vacuo*. A yield of 6.0 g. of 2-(*p*-methoxyphenyl)-3-butanone, $n^{20}D$ 1.5616, was obtained. This material gave a positive iodoform test and formed a semicarbazone melting at 177.5–180°. This semicarbazone is reported by Tiffeneau[12] to melt at 183–184°. If the reaction had occurred on the methyl group the compound formed would have been 1-(*p*-methoxyphenyl)-2-butanone. This compound would not give an iodoform test and forms a semicarbazone melting at 131–132°.[13]

2-(*p*-Methoxyphenyl)-3-keto-7-methoxy-1,2,3,9,10,10a-hexahydrophenanthrene (I).—To a solution of 1.2 g. of sodium in 65 ml. of dry methanol in a 500-ml. 3-necked flask were added 10 g. of 2-hydroxymethylene-6-methoxy-α-tetralone and 18.9 g. of 1-dimethylamino-2-(*p*-methoxyphenyl)-3-butanone methiodide. The mixture was stirred at room temperature in an argon atmosphere for 30 minutes and then refluxed 48 hours. The solution was then cooled to 2° and a solution of 10 g. of potassium hydroxide in 50 ml. of methanol added over a period of 20 minutes, keeping the temperature below 2°. The mixture was then warmed to room temperature with stirring and allowed to stand overnight, refluxed two hours, cooled and filtered. The product was washed with water, hot methanol and dried. The product was dissolved in boiling toluene, decolorized with Norite and crystallized. A yield of 7.5 g. (46%) of product, m.p. 187–188.5°, was obtained; λ_{max}^{alc} 223 mμ (*E* 17400); 241 mμ (*E* 11,250); 330 mμ (*E* 28,600).

Anal. Calcd. for $C_{22}H_{22}O_3$: C, 79.06; H, 6.58. Found: C, 78.91; H, 6.59.

2-(*p*-Methoxyphenyl)-7-methoxy-1,2,3,4,9,10-hexahydrophenanthrene (II) and 2-(*p*-Methoxyphenyl)-7-methoxy-1,2,3,9,10,10a-hexahydrophenanthrene (III).—Compound I was reduced using the modification of the Wolff–Kishner technique outlined previously.[1] The solvent extracts were concentrated and crystallized from alcohol solution. The crude product was decolorized with Norite in benzene solution and recrystallized from hot acetone to give 1.0 g. (26%) of II, m.p. 148–151°; λ_{max}^{alc} 218 mμ (*E* 29,900), 272.5 mμ (*E* 21,400).

Anal. Calcd. for $C_{22}H_{24}O_2$: C, 82.50; H, 7.50. Found: C, 82.32; H, 7.63.

By evaporation of the mother liquors 1.4 g. (36%) of crude III, m.p. 103–108° could be isolated. By chromatographing on alumina from benzene–ligroin solution, followed by elution with benzene and recrystallization from alcohol, 0.3 g. of product melting at 121–123.5°, λ_{max}^{alc} 264.5 mμ (*E* 22,850), was obtained.

Anal. Calcd. for $C_{22}H_{24}O_2$: C, 82.50; H, 7.50. Found: C, 82.49; H, 7.55.

2-(*p*-Methoxyphenyl)-7-methoxy-1,2,3,4,4a,9,10,10a-octahydrophenanthrene (IV).—A solution of 1.9 g. of a mixture of II and III, m.p. 110–150°, in 30 ml. of sulfur-free benzene was hydrogenated at atmospheric pressure over 1.0 g. of 30% palladium–charcoal catalyst until gas absorption ceased. Approximately one mole of hydrogen was absorbed in 5 hours. The mixture was filtered and the filtrate evaporated to dryness *in vacuo*. The residue was recrystallized from acetone to give 1.6 g. (90%) of IV, m.p. 91.5–93°.

Anal. Calcd. for $C_{22}H_{26}O_2$: C, 81.98; H, 8.08. Found: C, 81.70; H, 8.13.

2-(*p*-Hydroxyphenyl)-7-hydroxy-1,2,3,4,4a,9,10,10a-octahydrophenanthrene (V).—The ether, IV, 1.1 g., was cleaved by refluxing in 48% hydrobromic acid–acetic acid solution and the reaction mixture worked up as previously described.[1] The crude product was dissolved in reagent ether, decolorized with Norite, and boiled with benzene until crystallization ensued. The product was separated and sublimed *in vacuo* to give 0.8 g. of V, m.p. 194–196° (vac.).

Anal. Calcd. for $C_{20}H_{22}O_2$: C, 81.63; H, 7.48. Found: C, 81.66; H, 7.64.

2-(*p*-Methoxyphenyl)-7-methoxy-1,2,3,4-tetrahydrophenanthrene (VI).—A solution of 1.2 g. of a mixture of II and III in 75 ml. of cyclohexene was placed in a glass liner with 0.8 g. of 30% palladium–charcoal catalyst and heated in a high-pressure reaction vessel, in an argon atmosphere, at 110° for 3 hours. The reaction mixture was filtered and the filtrate evaporated. The residue was recrystallized twice from hot butanone to give 0.5 g. (80%) of VI, m.p. 170.5–172.5°.

Anal. Calcd. for $C_{22}H_{22}O_2$: C, 83.02; H, 6.91. Found: C, 83.00; H, 7.17.

2-(*p*-Hydroxyphenyl)-7-hydroxy-1,2,3,4-tetrahydrophenanthrene (VII).—The ether VI, 0.9 g., was cleaved by heating with 25 g. of pyridine hydrochloride and the reaction mixture worked up as previously described.[1] The crude reaction product was dissolved in acetone, decolorized twice with Norite and the solution concentrated and chilled. A yield of 0.48 g. of VII was obtained, m.p. 228.5–230.3° (vac.).

Anal. Calcd. for $C_{20}H_{18}O_2$: C, 82.76; H, 6.21. Found: C, 82.92; H, 6.50.

2-(*p*-Methoxybenzyl)-6-methoxy-α-tetralone (VIII).—A mixture of 17.6 g. of 6-methoxy-α-tetralone, 3.9 g. of sodamide and 70 ml. of dry benzene was refluxed with stirring in a 300-ml. 3-necked flask in an argon atmosphere for 15 hours. The mixture was cooled to 5°, diluted with 50 ml. of dry ether, and a solution of 15.6 g. of *p*-methoxybenzyl chloride in 25 ml. of dry ether added rapidly. The mixture was allowed to warm slowly to room temperature and then

(9) E. Knoevenagel and L. Walter, *Ber.*, **37B**, 4502 (1904).

(10) A. C. Cope, C. H. Hofmann, C. Wyckoff and E. Hardenbergh, THIS JOURNAL, **63**, 3452 (1941).

(11) H. B. Hass, A. G. Susie and R. L. Heider, *J. Org. Chem.*, **15**, 8 (1950).

(11a) Heilbron, "Dictionary of Organic Compounds."

(12) M. Tiffeneau, J. Lévy and P. Weill, *Bull. soc. chim.*, **49**, 1709 (1931).

(13) J. Lévy and Dvoleitzka-Gombinska, *ibid.*, **49**, 1765 (1931).

heated at reflux for two hours. The mixture was cooled, diluted with water and the solvent layer separated, washed with dilute potassium hydroxide, water and dried over magnesium sulfate. The mixture was filtered and the filtrate concentrated *in vacuo*. The crystalline material that separated from the residue on cooling was washed with cold alcohol, decolorized with Norite in benzene, 60° naphtha solution and recrystallized twice from ethanol to give 14.0 g. (47%) of VIII, m.p. 91.5–93°; λ_{max}^{alc} 225 mμ (*E* 24,450), 276 mμ (*E* 19,500).

Anal. Calcd. for $C_{19}H_{20}O_3$: C, 77.03; H, 6.75. Found: C, 77.14; H, 6.56.

1-Ethyl-2-(*p*-methoxybenzyl)-6-methoxy-3,4-dihydronaphthalene (IX).—To a Grignard reagent made from 3.2 g. of ethyl bromide and 0.9 g. of magnesium turnings in 25 ml. of dry ether was added a solution of 6.0 g. of VIII in 30 ml. of dry benzene. The reaction was carried out at −5° in an argon atmosphere. After warming to room temperature and allowing to stand overnight, the reaction mixture was hydrolyzed and the product extracted with ether. On evaporating the solvent and distilling the residue, a yield of 5.1 g. (77%) of IX distilling at 180–197° (0.1 mm.), was obtained.

Anal. Calcd. for $C_{21}H_{24}O_2$: C, 81.82; H, 7.79. Found: C, 81.66; H, 7.82.

1-Ethyl-2-(*p*-methoxybenzyl)-6-methoxy-1,2,3,4-tetrahydronaphthalene (X).—A solution of 5.0 g. of IX in 50 ml. of sulfur-free benzene and 10 ml. of *n*-propanol was hydrogenated over 0.5 g. of 30% palladium–charcoal catalyst at atmospheric pressure until hydrogen uptake ceased. Approximately one mole of hydrogen was absorbed in six hours. The mixture was filtered and the filtrate evaporated and distilled *in vacuo*. A yield of 5.0 g. of X distilling at 175–177° (0.1 mm.) was obtained.

Anal. Calcd. for $C_{21}H_{28}O_2$: C, 81.29; H, 8.38. Found: C, 81.17; H, 8.32.

1-Ethyl-2-(*p*-hydroxybenzyl)-6-hydroxy-1,2,3,4-tetrahydronaphthalene (XI).—The ether X, 1.5 g., was cleaved by refluxing in 48% hydrobromic acid–acetic acid solution and the reaction mixture worked up as previously described.[1] The product was purified using the method employed with compound V. A yield of 1.1 g. of XI, m.p. 116–120°, was obtained.

Anal. Calcd. for $C_{19}H_{22}O_2$: C, 80.85; H, 7.80. Found: C, 80.94; H, 7.70.

The 1-Ethyl-2-(*p*-methoxybenzyl)-6-methoxynaphthalene (XII).–A mixture of 3.0 g. of X and 0.2 g. of 30% palladium–charcoal catalyst was heated to 210° in an argon atmosphere. The evolution of hydrogen slowed in 20 minutes and heating was continued for 10 minutes more. The mixture was cooled, taken up in ether, and filtered. The filtrate was concentrated, the residue dissolved in warm alcohol, treated with 2 g. of picric acid and the solution cooled to give 4.2 g. (81%) of the picrate, m.p. 80–81.5°. The picrates from several similar runs were combined and recrystallized from ethanol to give a purified product melting at 82–82.5°.

Anal. Calcd. for $C_{27}H_{25}O_9N_3$: N, 7.85. Found: N, 7.76.

Picrate totalling 8.8 g. was dissolved in benzene and passed through a column containing 22 g. of activated alumina (Fisher), and the column washed with benzene until the yellow zone approached the bottom. The eluate was concentrated and the residue distilled *in vacuo*. The distillate was recrystallized from methanol to give 3.8 g. (77%) of product, m.p. 71.2–72.5°.

Anal. Calcd. for $C_{21}H_{22}O_3$: C, 82.35; H, 7.19. Found: C, 82.52; H, 7.36.

The 1-Ethyl-2-(*p*-hydroxybenzyl)-6-hydroxynaphthalene (XIII).—The ether XII, 2.0 g. was cleaved by heating with 30 g. of pyridine hydrochloride and the reaction mixture worked up as previously described.[1] The crude product was boiled with benzene–60° naphtha solution to form a solid which was dissolved in reagent ether, decolorized twice with Norite, benzene added and the solution evaporated *in vacuo* until a solid separated. A yield of 1.0 g. of product, which darkened on exposure to air, was obtained, m.p. 164–166° (vac.).

Anal. Calcd. for $C_{19}H_{18}O_2$: C, 82.01; H, 6.47. Found: C, 82.14; H, 6.56.

MISSOULA, MONTANA

NOTES

A Specific Test Differentiating between α-Ketol and Dihydroxyacetone Groups of C_{21}-Steroids on Paper Chromatograms

BY L. R. AXELROD

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The use of alkaline triphenyltetrazolium chloride solution to detect any side-chain containing a C_{17} α-ketol has had wide application in the chromatography of adrenal steroids.[1] This test however does not differentiate between the α-ketol side-chain and one which, in addition, contains a tertiary hydroxyl group at the C_{17}-position. If with the use of the above reagent a characteristic red spot appears on a strip from a paper chromatogram, the following test may then be applied to demonstrate the presence or absence of a dihydroxyacetone group (*i.e.*, an α-ketol with a C_{17} tertiary hydroxyl group).

Another strip from the same chromatogram is passed through aqueous 0.1 *N* NaOH and placed on a glass plate which has been heating on a Lindberg hot plate (surface temperature, 100°). The strip is covered with another glass plate and the heating continued for exactly three minutes after which time the topmost plate is removed and the strip allowed to dry on the heated plate. The strip is then passed through the usual alkaline triphenyltetrazolium solution and returned to the heated glass plate until maximum color production (about 15 sec.).

The appearance of a red color in the same position as on the first strip is evidence for an α-ketol side-chain without the added tertiary hydroxyl group, whereas no red color will appear if a dihydroxyacetone structure is present. This test is based on an observation by Mason, *et al.*,[2] that the dihydroxyacetone side-chain is very labile to dilute alkali, whereas the α-ketol is much more stable.

(1) R. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, **188**, 763 (1951).

(2) H. L. Mason, W. M. Hoehn and E. C. Kendall, *ibid.*, **124**, 459 (1938).