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 $C_{21}H_{26}O_2$: 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

RECEIVED MAY 5, 1953

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.

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[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).

Under the conditions of the above spot test, up to 40–50 γ of a steroid with a dihydroxyacetone side-chain per 1 cm. width of chromatogram will be destroyed in the allotted time. Exposure to higher temperatures than 100–110° or concentrations of alkali greater than 0.1 *N* will destroy some of the α-ketol group. The appearance of a light yellow-pink color after the test has been executed, as compared to the vivid red on the first strip, should be taken as a positive test for the dihydroxyacetone group.

This test has been used successfully on chromatograms in differentiating the side-chains of cortisone, hydrocortisone, substance S of Reichstein, and substance D of Reichstein from corticosterone, desoxycorticosterone and allopregnane-3β,21-diol-20-one.

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Synthesis of Ethyl Ethoxybromoacetate and Ethyl Ethoxycyanoacetate[1]

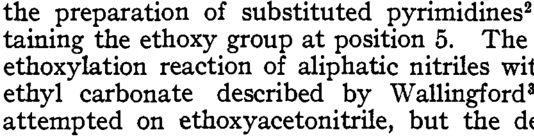
BY AARON BENDICH AND GRACE C. CLEMENTS

RECEIVED APRIL 15, 1953

Ethyl ethoxycyanoacetate (I) was required for the preparation of substituted pyrimidines[2] containing the ethoxy group at position 5. The carbethoxylation reaction of aliphatic nitriles with diethyl carbonate described by Wallingford[3] was attempted on ethoxyacetonitrile, but the desired ester I was obtained apparently together with its ethylated derivative ethyl α-ethoxy-α-ethylcyanoacetate. It has been pointed out previously[4] that in such carbethoxylation reactions, simultaneous alkylation by the alkyl carbonate often occurs.

The alternate route, described below, affords the ester I in good yield. For this method, ethyl ethoxybromoacetate (II) was prepared by direct bromination of ethyl ethoxyacetate in carbon tetrachloride solution. The bromo ester II possesses an extremely reactive bromo atom which is easily hydrolyzed on brief contact with cold water. It also readily produces iodine upon treatment with aqueous potassium iodide.

Several unsuccessful attempts were made to convert the bromo ester II to the cyano ester I upon treatment with either potassium or cuprous cyanide in methanol, ethanol, diethyl ether or benzene. A 55% yield of the cyano ester I was obtained upon refluxing a suspension of cuprous cyanide in a xylene solution of II.



II I

Experimental

Ethyl Ethoxybromoacetate.—Ethyl ethoxyacetate (66 g., 0.5 mole) was dissolved in 65 ml. of carbon tetrachloride. The solution was stirred and kept refluxing while 80 g. (0.5 mole) of bromine was added at a rate such as to prevent the presence of excess bromine. The addition of bromine required about 3 hours. The HBr that had formed was removed by aeration and the residue was fractionated *in vacuo*. The fraction boiling 82–93° at about 10 mm. was redistilled; b.p. 94–95° (15 mm.) (197° at 750 mm.). The yield was 79 g. (75%).

Anal. Calcd. for $C_6H_{11}O_3Br$: C, 34.14; H, 5.25; Br, 37.86. Found: C, 33.93; H, 5.61; Br, 37.63.

Ethyl Ethoxycyanoacetate.—Ethyl ethoxybromoacetate (15 g., 0.071 mole) was dissolved in an equal volume of xylene (b.p. 135°) and 13.6 g. of *cuprous cyanide* (0.076 mole of $Cu_2(CN)_2$ was added. The mixture was stirred vigorously and was refluxed for 5 hours at the end of which time it no longer released iodine upon treatment with aqueous potassium iodide. The insoluble salts were removed and the fluid was distilled *in vacuo*. The fraction boiling at 94–100° at 11 mm. was collected; yield 6.2 g. (55%). It was refractionated: b.p. 95.0–96.5° (11 mm.), 217–218° (750 mm.).

Anal. Calcd. for $C_7H_{11}O_3N$: C, 53.48; H, 7.05; N, 8.91; total OC_2H_5, 57.33. Found: C, 53.46; H, 7.05; N, 8.81; total OC_2H_5, 57.22.

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[3] V. H. Wallingford, D. M. Jones and A. H. Homeyer, THIS JOURNAL, **64**, 576 (1942).

[4] V. H. Wallingford, A. H. Homeyer and D. M. Jones, *ibid.*, **63**, 2056 (1941).

The Metabolic Fate of Imidazoleacetic($C^{14}OOH$) Acid in the Rat

BY L. P. BOUTHILLIER AND GILLES LÉVEILLÉ

RECEIVED APRIL 10, 1953

It has been shown conclusively that imidazoleacetic acid is a product of oxidation of histamine in the intact rat.[1–3] However, the metabolism of imidazoleacetic acid is as yet unknown and the question arises whether this substance can be broken down or not *in vivo*. In this paper, we wish to report the results of experiments which provide evidence that this metabolite does not undergo oxidation in the rat tissues. Single doses of imidazoleacetic ($C^{14}OOH$) acid were administered intraperitoneally to three rats. The respiratory carbon dioxide and urine were collected for a period of 24 hours and the radioactivity of each was determined. Our experimental data, summarized in Table I, show that nearly 90% of the injected radiocarbon was recovered as imidazoleacetic acid in the urine samples, through the use of the isotope dilution technique. However, no radioactivity could be measured in the expired carbon dioxide. Employing the ascending method, one-dimensional paper chromatograms of urine samples were prepared in *n*-butanol–glacial acetic acid–

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[2] H. Tabor, A. H. Mehler and R. W. Schayer, *ibid.*, **200**, 605 (1953).

[3] L. P. Bouthillier and Moe Goldner, *Arch. Biochem. Biophys.*, in press.