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]

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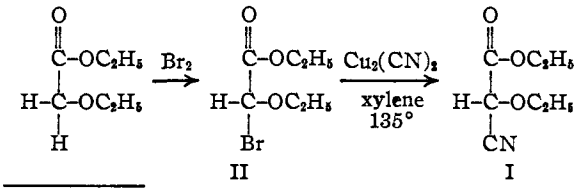
Ethyl ethoxycyanoacetate (I) was required for the preparation of substituted pyrimidines[2] containing the ethoxy group at position 5. The carbethoxylation reaction of aliphatic nitriles with diethyl carbonate described by Wallingford[3] was attempted on ethoxyacetonitrile, but the desired ester I was 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.



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

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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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