before another was added (time of addition, five hours). The reaction mixture was then poured into five volumes of water and the resulting precipitate was collected. Recrystallization from methylene chloride–hexane afforded 0.69 g. (49%) of VI, m.p. 186–188° dec. (with recrystallization at 115–120°), $[\alpha]^{25}D$ +44° (1% in chloroform).

Anal. Calcd. for $C_{23}H_{31}O_5Br$: Br, 17.09. Found: Br, 17.04.

16,17-Oxido-4-pregnen-11α-ol-3,20-dione Acetate (VII).—To a solution of 0.5 g. of VI in 50 ml. of glacial acetic acid was added, under an atmosphere of carbon dioxide, a solution containing 272 mg. of semicarbazide hydrochloride, 195 mg. of anhydrous sodium acetate, 10 ml. of water and 10 ml. of glacial acetic acid. The mixture was agitated for ten minutes and there was then added 20 ml. of 1 *N* sodium acetate in glacial acetic acid. Agitation was continued for ten minutes longer, 2 ml. of pyruvic acid was added, and the mixture was refluxed for ten minutes. The cooled solution was diluted with water and extracted with methylene chloride. The extracts were washed free of acid with water, dried over magnesium sulfate and concentrated to a small volume. Hexane was then added to the point of opalescence and the solution was chromatographed on 20 g. of Florisil prepared with hexane. Elution with hexane and mixtures of hexane and ether stripped nothing from the column. From elution with ether there resulted five 50 ml. fractions containing a total of 0.103 g. (25%) of VII, m.p. 212–214°. Recrystallization from methylene chloride–hexane raised the m.p. to 217–218°, $[\alpha]^{25}D$ +112.9° (1% in chloroform).

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.55; H, 8.00.

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The Preparation of 2-C^{14}-Adenine

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As a preliminary to a study of the metabolism of the purines, with especial reference to the 2-position of the ring, the synthesis of adenine labeled in the 2-position with C^{14} was undertaken. The method described by Shaw,[1] in which 4-amino-5-imidazolecarboxamidine is formylated and the product cyclized to give adenine, appeared to be suitable since by using C^{14}-formic acid for the formylation 2-labeled adenine would be obtained. An advantage of this method is that the isotope would be introduced at a late step in the synthesis, thereby minimizing losses of radioactive material. The undesirable feature of the method, however, as far as economy of radioactive material is concerned, is that the formylation is carried out with a large excess of 98% formic acid in the presence of acetic anhydride. This would necessitate the use of an inordinately large amount of C^{14}-formate in order to obtain adenine with appreciable radioactivity.

In order to avoid the use of such a large excess of formic acid, experiments were carried out to study the feasibility of formylating the carboxamidine with an aqueous solution of formic acid, since such conditions have been used to formylate other amines.[2,3] The formylation reaction was found to proceed in 6 *M* formic acid, and by using this modification it was possible to obtain 2-C^{14}-adenine in yields of 60–65%, based on the carboxamidine used. The unreacted C^{14}-formate can be recovered almost quantitatively and used for further preparations of labeled adenine.

Method.—A solution of 0.200 g. of 4-amino-5-imidazolecarboxamidine dihydrochloride[1] in 2.0 ml. of 20% formic acid was placed in a reaction tube made from the outer member of a 24/40 standard taper joint. To this solution was added 0.170 g. of potassium formate, making the solution 6.3 *M* with respect to formate. The solution was then boiled gently under reflux for 4 hours. The formamido derivative was not isolated but was cyclized to adenine by diluting the solution to 8 ml. with water, adding sufficient potassium bicarbonate to neutralize the formic acid and to make the solution 0.5 *M* in bicarbonate, and then boiling under reflux for 1 hour. An amount of hydrochloric acid slightly less than that required to neutralize the solution was added, and the solution was concentrated under reduced pressure to a volume of 2–3 ml. On placing the solution in the refrigerator for several hours crude adenine precipitated. This material was collected by centrifugation, washed 3 times with ice-cold water and dried *in vacuo*. The supernatant and wash liquids were saved for the recovery of unreacted formate. The crude material was sublimed at 220° and a pressure of 1 mm. to give 0.083 g. of pure adenine, a yield of 61% based on the carboxamidine. Yields of 40–42% were obtained when the formylation was carried out with 4.0 *M* formic acid.

Anal. Calcd. for $C_5H_5N_5$: C, 44.44. Found: C, 44.27.

The compound formed a picrate which melted with decomposition at 286–287°.[1] Admixture with picrate prepared from authentic adenine did not depress the m.p. The ultraviolet absorption spectrum and R_f values obtained by paper chromatography[4] were identical with those of authentic adenine.

2-C^{14}-Adenine was prepared by using C^{14}-potassium formate in the above procedure. In a typical experiment, adenine having a specific activity of 1.055×10^6 c.p.m. per m*M* was synthesized and the formate recovered from the reaction mixture had a specific activity of 1.025×10^6 c.p.m. per m*M*.

The unreacted C^{14}-formate in the supernatant fluid and washings after separation of the crude adenine was recovered almost quantitatively by steam distillation.[5] For further use in preparing radioactive adenine, the steam distillate was titrated with standard potassium hydroxide solution and concentrated to small volume under reduced pressure. The concentrate was then transferred to the reaction tube and evaporated to dryness. The appropriate amount of 4-amino-5-imidazolecarboxamidine dihydrochloride was added, followed by hydrochloric acid equivalent to the formate present less the amount of hydrochloric acid present as the dihydrochloride salt. The procedure outlined above was then followed for the remainder of the synthesis.

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The Tetrachlorophthalic Anhydride Derivatives of Some Alkylbenzenes

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In a previous note[2] the authors reported the phthalic anhydride derivatives of several substituted alkylbenzenes. In general these derivatives distinguish satisfactorily among the alkylbenzene hydrocarbons. However, it is not possible by

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