EXPERIMENTAL

Materials. The aryl chloroformates were synthesized in this laboratory by the method of Raiford and Inman.[7] The aromatic hydrocarbons used were Eastman grade. The aluminum chloride was Baker and Adamson, anhydrous, Reagent grade.

Phenyl benzoate. A mixture of 2.0 g. (0.0128 mole) of phenyl chloroformate, 1.75 g. (0.013 mole) of aluminum chloride and 10 cc. of dry benzene was heated on the steam bath under reflux for 1 hr. At the end of this time the evolution of gaseous hydrogen chloride had practically ceased. In all the preparations described it was assumed that the reaction was complete when gas was no longer evolved from the reaction mixture. After cooling to room temperature the reaction mixture was added slowly with stirring to an excess of ice-cooled dilute hydrochloric acid solution. After decomposition of the complex was complete the benzene layer was washed with water and then 5% sodium hydroxide solution. No appreciable precipitate formed when the alkaline extract was acidified. This was an indication that the aryl ester had not undergone the Fries rearrangement to form a hydroxyaryl ketone. The benzene was removed by steam distillation. The nonvolatile oil solidified when the water-oil mixture was cooled in ice, m. p. 66–68°. A mixed melting point of this product with a known sample of phenyl benzoate showed no depression. The yield was 1.62 g. (64%).

p-Chlorophenyl benzoate. A mixture of 3.82 g. (0.020 mole) of *p*-chlorophenyl chloroformate, 3.0 g. (0.0225 mole) of aluminum chloride and 20 cc. of dry benzene was refluxed on the steam bath for 5 hr. The reaction product was decomposed with cold dilute hydrochloric acid as previously described. To aid in solubilizing the product in the benzene layer 20 cc. of diethyl ether was added. The benzene-ether layer was extracted with 5% sodium hydroxide solution and then washed with water. After removal of the benzene and ether by steam distillation the nonvolatile oil solidified when the water-oil mixture was cooled to room temperature. The crude product was crystallized from ethyl alcohol using charcoal, m.p. 88–89°. The yield was 2.7 g. (58%). There was no depression of the melting point when the product was mixed with a known sample of *p*-chlorophenyl benzoate which had been prepared by the Schotten-Baumann reaction.

p-Phenylphenyl benzoate. A mixture of 11.6 g. (0.05 mole) of *p*-phenylphenyl chloroformate, 8.0 g. (0.06 mole) of aluminum chloride and 30 cc. of dry benzene was heated on the steam bath under reflux for 45 min. The cooled reaction mixture was poured, with stirring, into an excess of cold dilute hydrochloric acid. A rather viscous mass resulted after stirring for 5 hr. After steam was passed through this mixture to remove excess benzene the nonvolatile oily residue solidified when cooled to room temperature. The crude product was crystallized from ethyl alcohol, m.p. 147–148°. A mixed melting point with a known sample of *p*-phenylphenyl benzoate showed no depression. The yield was 7.0 g. (51%).

Anal. Calcd. for $C_{19}H_{14}O_2$: C, 83.21; H, 5.11. Found: C, 83.23. 83.10; H, 5.08, 4.96.

p-Phenylphenyl p-toluate. A mixture of 11.6 g. (0.050 mole) of *p*-phenylphenylchloroformate, 8.0 g. (0.06 mole) of aluminum chloride and 35 cc. of dry toluene was refluxed for 1 hr. on the steam bath. The cooled reaction mixture was decomposed with dilute hydrochloric acid. After removing the excess toluene by steam distillation it was found that the nonvolatile oil solidified when the water-oil mixture was cooled. This solid was extracted with diethyl ether and the ethereal solution dried with potassium carbonate. The product resulting from the removal of the ether was crystallized from ethyl alcohol, m.p. 131–133°. The yield was 4.2 g. (30%). *p*-Phenylphenol and *p*-toluic acid were identified as the products of hydrolysis of the above substance with alcoholic potassium hydroxide.

Anal. Calcd. for $C_{20}H_{16}O_2$: C, 83.33; H, 5.56. Found: C, 83.19, 83.18; H, 5.26, 5.38.

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Degradation of 3α,17α,21-Trihydroxypregnan-20-one-C^{14} Biosynthesized from Acetate-1-C^{14} by a Cushing's Patient[1,2]

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In a previous communication[3] we reported the distribution of radioactivity in carbons 20 and 21 of cortisol-C^{14} biosynthesized from acetate-1-C^{14} by perfusion of the isolated calf adrenal gland. It was found that carbon 20 was derived from the carboxyl carbon and carbon 21 from the methyl carbon of acetate. This finding agreed with the scheme of Woodward and Bloch[4] for the incorporation of acetate into cholesterol. This communication deals with a similar study on a C^{14}-labeled adrenocortical steroid isolated from the urine of a human following the administration of acetate-1-C^{14}.[5]

Since there was not sufficient nonlabeled material available to serve as carrier for the degradation, the radioactive steroid, 3α,17α,21-trihydroxypregnan-20-one, was diluted with a related substance with a dihydroxy acetone side chain, 17α,21-dihydroxy-4-pregnene-3,20-dione. The mixture of 3α,17α,21-trihydroxypregnan-20-one-C^{14} and 17α,-21-dihydroxy-4-pregnene-3,20-dione had a corrected specific activity of 80.5 × 10^3 counts/min./mM. The mixture was reduced with sodium borohydride and subsequently oxidized with periodic acid to

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[2] Permission was granted by the Atomic Energy Commission to administer 2 mc of acetate-1-C^{14} to a female patient in the terminal stages of adrenal cortical carcinoma. The cooperation of George W. Thorn and staff at the Peter Bent Brigham Hospital, Boston, is gratefully acknowledged.

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yield formaldehyde from C-21 and formic acid from C-20. The formaldehyde was precipitated as formadone and the formic acid was oxidized with mercuric oxide to carbon dioxide. The carbon dioxide was trapped in carbonate-free sodium hydroxide and subsequently precipitated as barium carbonate. No significant counts could be detected in the formadone which indicated that less than 50 counts/min./mM could have been present. On the other hand, 7.77×10^3 counts/min./mM were found in the barium carbonate.

The distribution of radioactivity in carbons 20 and 21 in $3\alpha,17\alpha$,21-trihydroxypregnan-20-one-C^{14} agrees with that obtained in cortisol-C^{14} by calf adrenal perfusion.[8] On the basis of the scheme postulated by Woodward and Bloch[4] we would expect ten radioactive carbons to be incorporated into the first 21 carbons of cholesterol from acetate-1-C^{14}. If one assumes that corticosteroids are biosynthesized from acetate-1-C^{14} through cholesterol then the arrangement of all carbons of C^{14} atoms in corticosteroids would be expected to be the same as that found in the first 21 carbons of cholesterol. The present findings support the view that the carboxyl and the methyl carbons of acetate are incorporated into carbon 20 and 21, respectively, of a corticosteroid. The distribution of methyl and carboxyl carbons in the corticoid side chain is identical to that of carbons 20 and 21 of cholesterol.

EXPERIMENTAL

The specific activities were determined by the method of Karnovsky *et al.*[6] using a Robinson proportional counter.

Isolation and purification of C^{14}-labeled $3\alpha,17\alpha$,21-trihydroxypregnan-20-one. Following the administration of 2 mc. of 1-C^{14}-acetate to a female patient[2] the urine was collected for 5 days.[5] The urine was hydrolyzed with β-glucuronidase and extracted with ethyl acetate. The ketonic fraction, prepared by a modified Girard separation,[7] was chromatographed on a silica gel column. The crystalline material eluted with benzene-ethyl acetate (3:1) was rechromatographed in the same system. Repeated recrystallization from acetone and finally ethanol gave crystals melting at 197–204° with a constant specific activity.

Specific activity determination of carbons 20 and 21. Chromatographically pure unlabeled 17α,21-dihydroxy-4-pregnene-3,20-dione (211 mg.) was added as carrier to the C^{14}-labeled $3\alpha,17\alpha$,21-trihydroxypregnan-20-one (32.6 mg.) described in the section above. The specific activity of the mixture was 80.5×10^3 counts/min./mM. A 103 mg. portion of the mixture was dissolved in 15 ml. of methanol and stirred overnight at room temperature with 500 mg. of sodium borohydride. Water was added and following acidification with hydrochloric acid the mixture was extracted with ethyl acetate. The extract was washed with water, a saturated solution of sodium bicarbonate, water, dried over sodium sulfate, and taken to dryness *in vacuo*. The residue (108 mg.) was dissolved in 25 ml. of carbon dioxide–free ethanol, 36 ml. of a stock periodate solution were added and the solution was left for 135 min. at room temperature in the dark. The stock periodate solution was prepared by dissolving 1 g. of sodium metaperiodate in 70 ml. of water, adjusting the *p*H to 4 with 2*N* sulfuric acid, and making up the volume to 100 ml. The reaction mixture was distilled *in vacuo* to dryness and the distillate was collected in two flasks cooled with a dry ice–acetone mixture. The contents of both flasks were combined (80 ml.).

Oxidation of formic acid to carbon dioxide. A portion of the distillate (55 ml.) was flushed for 5 min. with oxygen to expel carbon dioxide; 2 g. of red mercuric oxide were added, the oxygen stream continued, and the mixture boiled for 30 min. The evolving gases were collected through a reflux condenser into two traps containing carbon dioxide-free sodium hydroxide. The collected carbon dioxide was precipitated as barium carbonate[8] and counted.

Precipitation of formadone. To a portion (25 ml.) of the distillate obtained following periodic oxidation, 1 ml. of glacial acetic acid was added and the solution was buffered with 300 mg. of sodium acetate. The formaldehyde was precipitated with dimedone[9] and the crystals collected, m.p. 193–194°. The formadone was recrystallized from methanol to a constant specific activity.

For the specific activity determinations blank experiments were carried out and the appropriate corrections were introduced.

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Electrical Effect of the Triphenylmethyl Group on an Aromatic Ring

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The rates of the reactions of *meta*- and *para*-tritylbenzoic[1] acids with diphenyldiazomethane and those of *meta*- and *para*-tritylphenoxides with ethyl iodide have been obtained. These were treated in the usual manner to obtain the values of Hammett's "Substituent Constant[2] (σ)" for the triphenylmethyl group.

The *meta*- and *para*-tritylbenzoic acids were obtained by the oxidation of the corresponding tolyl compounds.[3]

Para-tritylphenol was prepared by the tritylation of phenol. *Meta*-tritylphenol was synthesized

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[1] "Trityl" has been used as a trivial name for the triphenylmethyl group.

[2] L. P. Hammett, *Physical Organic Chemistry*, McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 184.

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