the egg albumin was found to be degraded by the treatment. Similar results have been obtained by Reyerson and Peterson working with insulin.[7]

Similarly, in the cases of the proteins egg albumin, β-lactoglobulin, lactalbumin, zinc insulin, fibrin, casein and bovine plasma albumin the amount of BF_3 bound permanently varied with temperature in the range 0° to 80°. In the first four cases, the amount of BF_3 bound decreased to zero at 80°. Equilibrium at the higher temperatures was achieved only after several days, and at the same time the proteins were observed to undergo degradation and loss in weight. Details of these studies will be published separately.

These results indicate that despite the striking correlations obtained between functional groupings and protein–HCl complexing, the agreements may be fortuitous. This question can only be resolved by a very careful study of the desorption isotherms of the protein–HCl complexes in the low pressure region over an extended temperature range.

To study this point further, isotherms for the desorption of HCl from egg albumin were studied at 32 and at 52°. HCl at a pressure of 10 cm. was sorbed on a sample of spray-frozen egg albumin and the desorption was followed stepwise using a versatile range, combination gas buret and McLeod gage (range 3 to 10^{-5} cm.) to measure gas volumes and pressures. Equilibrium was reached in 6–10 hours. At pressures below 10^{-4} cm. the outgassing of the walls became troublesome and for this reason the isotherms were not followed below this pressure.

Figure 1 shows the isotherms at 32 and 52°. The latter shows clearly an isobaric region in the neighborhood of 0.1 mm. which can be interpreted as evidence for compound formation. Since only one point was taken in this region, the extent of the isobar cannot be determined accurately. While there is no such isobar present in the 32° desorption isotherm, its presence can be reasonably inferred from the shape of the curve and the knowledge (from other work) that an asymptote exists at about 10^{-4} to 10^{-5} cm. and 0.95 ± 0.05 mmole HCl/g. protein.

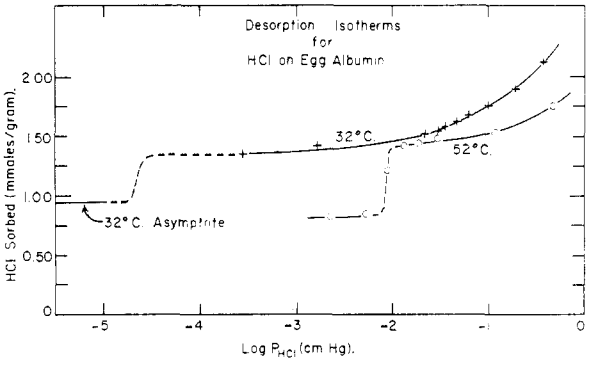

Fig. 1.—Desorption isotherms for HCl on egg albumin: +, experimental points at 32° (upper curve); solid line at left of upper curve is experimental asymptote at 32°; dashed line is hypothetical; ⊙, refer to experimental points at 52°; dashed line is hypothetical.

Using the Clausius–Clapeyron equation, the partial molal heats of binding are found to have values of 15.8 kcal./mole for the HCl sorbed above the isobaric region and a minimum value of 43 kcal./mole for the HCl bound below the isobaric region. This latter value indicates that the "permanently" bound HCl which has been attributed to strongly binding basic groups is bound as strongly as the HCl in such hydrochlorides as NH_4Cl. This lends further support to the identification of this fraction as stoichiometrically bound HCl.

This preliminary work appears very promising since a study of the desorption isotherms appears to offer an unambiguous method of investigating the nature of the compounds of HCl with proteins. It is possible that the free basic groups can be distinguished among themselves in this manner. The chief difficulty with the method which is not insuperable is the long equilibration time. In principle, the method can be extended to lower pressures by correcting properly for the outgassing from the walls.

(7) L. H. Reyerson and L. Peterson, *J. Phys. Chem.*, **59**, 1117 (1955).

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Preparation of Flavanone-2-C^{14} by an Exchange Reaction[1]

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The preparation of flavanones by the isomerization of the corresponding 2′-hydroxychalcones is a well-established procedure.[3] Polyhydroxychalcones usually have been prepared by the condensation of the appropriately substituted benzaldehyde with the required *o*-hydroxyacetophenone in the presence of a basic catalyst.[4] Although the isomerization reaction is usually quite efficient,[5] the condensation reaction to produce the chalcone is frequently poor for the more highly hydroxylated compounds.[6]

Since work here and elsewhere[7] has demonstrated the reversibility of the benzaldehyde–acetophenone condensation, a simple path to C^{14}-labeled flavanones, which might avoid the low yields in the condensation step, appeared to be the exchange of the appropriate carbonyl-labeled benzaldehyde with the desired non-radioactive chalcone followed by ring-closure of the recovered labeled chalcone.

As a test, equimolar amounts of benzaldehyde-α-C^{14} and *o*-hydroxybenzylideneacetophenone were

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(3) (a) S. v. Kostanecki and W. Szabranski, *Ber.*, **37**, 2635 (1904); (b) F. Mayer and A. H. Cook, "The Chemistry of Natural Coloring Matters," Reinhold Publishing Corp., New York, N. Y., 1943, p. 164.

(4) (a) C. Feuerstein and S. v. Kostanecki, *Ber.*, **31**, 715 (1897); (b) T. Emilewicz and S. v. Kostanecki, *ibid.*, **28**, 2014 (1898).

(5) L. Reichel and J. Steudel, *Ann.*, **553**, 83 (1942).

(6) A. V. Balaiah, L. R. Row and T. R. Seshadri, *Proc. Indian Acad. Sci.*, **20A**, 274 (1944).

(7) (a) R. L. Frank and R. P. Seven, THIS JOURNAL, **71**, 2629 (1949); (b) M. Weiss, *ibid.*, **74**, 200 (1952).

allowed to exchange in basic solution. The recovered (90%) *o*-hydroxy-(benzylidene-α-C^{14})-acetophenone was isomerized to flavanone-2-C^{14} in 80% yield. Since future work will require more highly hydroxylated compounds rather than flavanone itself, no effort was devoted to finding the best conditions for complete exchange, nor in discovering the best recovery methods. It is obvious that the efficiency of the radioactivity transfer would be improved by use of a smaller molar proportion of the benzaldehyde-α-C^{14} and by carrying out the exchange under such conditions that by-product formation is minimized.

Experimental

2'-Hydroxy-(benzylidene-α-C^{14})-acetophenone.—A 0.5-g. portion of benzaldehyde-α-C^{14}, whose millimolar activity was 1.49 microcuries, was added to a cooled solution of 1.1 g. of 2'-hydroxybenzylideneacetophenone,[4a] 2 ml. of 20% sodium hydroxide and 20 ml. of ethanol. The solution was stirred for 60 hours at room temperature, diluted with water and acidified. The 2'-hydroxy-(benzylidene-α-C^{14})-acetophenone which separated was recrystallized twice from methanol to give 0.98 g. (90%) of recovered product, m.p. 86°, whose millimolar activity was 0.130 microcurie.[8]

Flavanone-2-C^{14}.—A 0.5-g. sample of the 2'-hydroxy-(benzylidene-α-C^{14})-acetophenone was dissolved in 30 ml. of ethanol containing 1 ml. of 1% sodium hydroxide. The solution was stirred at room temperature for 24 hours, adjusted to *p*H 6 with dil. hydrochloric acid, and diluted with water.

The product which precipitated from the chilled solution weighed 0.4 g. (80%), m.p. 74°,[3a] after five crystallizations from methanol; radioactivity, 0.130 μc./mmole.

(8) Radioactivity measurements were made by wet combustion of 10- to 20-mg. samples of organic compounds to carbon dioxide and determination of the ion current with a vibrating-reed electrometer; *cf.* O. K. Neville, THIS JOURNAL, **70**, 3501 (1948).

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2-Isopropyl-1-naphthol

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In 1929, Meyer and Bernhauer[1] reported the preparation of a compound which they described as 2-isopropyl-1-naphthol (m.p. 65–66°, benzoate ester, m.p. 121). This material was obtained by the potassium hydroxide fusion of a sulfonic acid prepared by the sulfonation of 2-isopropylnaphthalene.

We have recently had occasion to synthesize 2-isopropyl-1-naphthol by a less ambiguous method and obtained a product, m.p. 47–48°, which yielded a benzoate, m.p. 67–68°. The synthesis is also of interest in that several new compounds were prepared as intermediates.

Experimental

Diethyl Isopropyl-(β-phenylethyl)-malonate (I).—Two experiments were made to obtain an adequate supply of this compound. In the first batch, a sodium dispersion was prepared from 11.5 g. (0.5 mole) of sodium and 200 ml. of toluene. The dispersion was stirred and maintained at 25–35° while a solution of 101 g. (0.5 mole) of diethyl isopropylmalonate[2] in 200 ml. of toluene was added. To the resultant slurry was then added 92.5 g. (0.5 mole) of β-phenylethyl bromide. The mixture was heated and became homogeneous at 71° and began to precipitate sodium bromide at 99°. Titration of a sample with acid after heating for one hour at 100° indicated 65% reaction. The mixture was heated at reflux for an additional four hours. The product was washed with water, a few drops of acetic acid being used in the final wash to remove traces of base. The toluene and less volatile materials were distilled, the distillation being stopped when the pot temperature reached 140° (17 mm.). The residue was then distilled from a Claisen flask to yield 43 g. (0.14 mole, 28% theory), of I, b.p. 120–130° (1–2 mm.), n^{20}D 1.4857.

Anal. Calcd. for $C_{18}H_{26}O_4$: C, 70.56; H, 8.55. Found: C, 70.0; H, 8.51.

A second run using twice the quantities employed above yielded 65 g. (0.21 mole, 21% theory) of I.

Ethyl 2-Isopropyl-4-phenylbutyrate (II).—This compound was produced inadvertently by the partial hydrolysis and decarboxylation of I. A solution of 100 g. (0.33 mole) of I and 271 ml. of 2.66 *N* ethanolic potassium hydroxide was refluxed for seven hours. Water (150 ml.) was then added and the alcohol removed by distillation. The resulting aqueous solution was extracted with ether to remove traces of neutral organics, then acidified and the product separated by ether extraction. Fractionation through a helix-packed column yielded 34 g. (0.14 mole, 44% theory) of II boiling at 161° (17 mm.).

Anal. Calcd. for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found: C, 77.0; H, 9.28.

There was also obtained 23 g. of the corresponding acid III and 5 g. of a mixture of II and III.

2-Isopropyl-4-phenylbutyric Acid (III).—The 34-g. portion of II and the 5-g. fraction of acid–ester mixture obtained in the above experiment were combined and refluxed with 50 ml. of 5.0 *N* 95% ethanolic potassium hydroxide for eight hours. Water was then added and the alcohol removed by distillation. Acidification of the aqueous solution yielded 23 g. of III. This material was combined with the acid obtained in the above experiment and distilled from a claisen flask to yield 52 g. of III, m.p. 56–58.5°, b.p. 168–170° (6 mm.). Recrystallization from isoöctane yielded a product having a melting point of 59.5–60°.

Anal. Calcd. for $C_{13}H_{18}O_2$: C, 75.69; H, 8.79. Found: C, 75.4; H, 8.75.

The anilide of III was prepared and upon recrystallization from isoöctane obtained as white crystals, m.p. 111–112°.

Anal. Calcd. for $C_{19}H_{23}NO$: C, 81.10; H, 8.24; N, 4.98. Found: C, 81.2; H, 8.49; N, 5.31.

2-Isopropyl-1-tetralone (IV).—The cyclization of III was effected by a procedure similar to that used by Adkins and Davis[3] for the synthesis of 2-methyl-1-tetralone.

The acid chloride of III was prepared by adding 52.2 g. (0.25 mole) of phosphorus pentachloride portionwise to 41.2 g. (0.2 mole) of III dissolved in 200 ml. of benzene. The mixture was refluxed for 30 minutes, cooled to and maintained at 25–30° while 104 g. (0.4 mole) of stannic chloride was added dropwise. After standing for 30 minutes the product was poured into 170 ml. of 12 *N* hydrochloric acid. The organic layer was washed three times with 4 *N* hydrochloric acid and then three times with 5% sodium carbonate solution. The product was dried by distilling off the benzene–water azeotrope and fractionated through a helix-packed column to yield 32 g. (0.17 mole, 85% theory) of 2-isopropyl-1-tetralone (IV), b.p. 162° (18 mm.), n^{20}D 1.4512.

Anal. Calcd. for $C_{13}H_{16}O$: C, 82.93; H, 8.57. Found: C, 82.5; H, 8.50.

The 2,4-dinitrophenylhydrazone of IV was prepared and recrystallized from ethanol as orange-red needles, m.p. 149–150°.

Anal. Calcd. for $C_{19}H_{20}N_4O_4$: C, 61.94; H, 5.47. Found: C, 61.9; H, 5.57.

2-Isopropyl-1-naphthol (V).—A mixture of 7.44 g. (0.040 mole) of 2-isopropyl-1-tetralone and 1.27 g. (0.040 mole) of sulfur was heated to 300° over a 10-minute period and maintained at 300 to 310° for four minutes. At the end of this time hydrogen sulfide evolution was virtually complete.

(1) H. Meyer and K. Bernhauer, *Monatsh.*, **53**, 721 (1929).

(2) C. S. Marvel and V. du Vigneaud, "Organic Syntheses," Coll. Vol. II, edited by A. H. Blatt, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 94.

(3) H. Adkins and J. W. Davis, THIS JOURNAL, **71**, 2955 (1949).