on the basis of the easy hydrolysis of N-formylanthranilic acid[7] and N′-formylkynurenine.[6] The product, Nα-acetylkynurenine, was then purified by ion-exchange chromatography and isolated in crystalline form.

Experimental

N-Acetyl-L-, -DL- and -D-tryptophan were prepared by acetylation of the corresponding optical isomers with acetic anhydride in sodium hydroxide.[8] Ozonolysis was carried out by the procedure of Warnell and Berg[6] with the ozone introduced through a coarse sintered glass disc, the ozone stream providing efficient stirring. One equivalent of ozone was thus passed into a suspension of 7.5 g. of finely powdered acetyltryptophan in 250 ml. of glacial acetic acid at room temperature. Hydrolysis to Nα-acetylkynurenine was effected by the addition of 30 ml. of concentrated hydrochloric acid to the reaction mixture which was then allowed to stand overnight at room temperature.

The hydrolyzed solution was concentrated to a thick sirup by vacuum distillation on a warm water-bath. The sirup was dissolved in 500 ml. of 0.1 *N* hydrochloric acid and applied to a column of Dowex-50 (Hydrogen form) 7 cm. in diameter and 12 cm. long. The column was then washed with 1.5 l. of 0.1 *N* HCl, 2.0 l. of 1.0 *N* HCl and 700 ml. of 2.4 *N* HCl at which time elution of the product began as indicated by the presence of a high concentration of diazotizable amine.[9] Elution with 2.4 *N* HCl was continued until the diazotizable amine concentration was negligible (about 4.0 l.). The amine fraction was cooled in an ice-bath and strong NaOH solution was added slowly with rapid stirring to adjust the *p*H to 2.5. The solution was saturated with sodium chloride and extracted with a total of 3 l. of ethyl acetate in six equal portions. The extract was vacuum distilled to dryness and the residue taken up in 150 ml. of warm absolute ethanol, treated with a small amount of decolorizing charcoal and filtered. Four volumes of petroleum ether (Skellysolve B) were added to the light yellow filtrate. Light golden-yellow crystals of acetylkynurenine appeared on standing overnight at −20°. The product was filtered, washed with a small volume of ether, and dried *in vacuo* (yield 20–50% of theoretical). The product was not hygroscopic and was quite stable. The acetyl-L- and acetyl-D-kynurenines were pale golden needles which crystallized readily from absolute ethanol–petroleum ether, whereas the acetyl-DL-kynurenine appeared as tiny rosettes which crystallized more slowly.

Anal. Calcd. for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.20. Found[10] for Nα-acetyl-L-kynurenine: C, 57.46, 57.49; H, 5.14, 5.29; N, 11.02, 11.20. Found for Nα-acetyl-DL-kynurenine: N, 10.96, 11.08. Found for Nα-acetyl-D-kynurenine: N, 11.09, 11.11.

The specific rotations and melting points are listed in Table I. The materials melted with foaming and decomposition at the indicated temperatures when the sample was inserted in the block at a temperature 15° below the melting point and then heated at the rate of 2° per minute.

TABLE I

Optical form	Sp. rotn. $[\alpha]^{25}D$ 0.5% in 95% ethanol	M.p. with dec., °C.
L	136.5°	188–188.5
DL	−0.3	171–171.5
D	−138.0	188–188.5

Paper chromatography revealed a single spot (bluish-green ultraviolet fluorescence) having an R_f of 0.82 (descending) in the organic phase of butanol, acetic acid, water 4:1:5, and R_f of 0.68 (ascending) in butanol, water, benzene, methanol 1:1:1:2. Hydrolysis in hydrochloric acid produced a different spot corresponding in R_f and ultraviolet fluorescence to kynurenine. Nα-Acetyl-DL-kynurenine did not resolve on paper whereas the hydrolysis product, DL-kynurenine resolved into spots of D- and L-kynurenine as described by Mason and Berg.[11] Nα-Acetyl-DL-kynurenine prepared by the procedure of Dalgleish[3] was a non-crystalline glass; however, this material crystallized readily from ethanol–petroleum ether as described above. The crystalline product was identical in melting point, chromatography on paper or Dowex-50 columns, and did not depress the melting point of Nα-acetyl-DL-kynurenine made from N-acetyl-DL-tryptophan as described above.

The ultraviolet absorption spectra of Nα-acetyl-L-kynurenine in acid, alkali and at neutrality were similar to those of kynurenine. At *p*H 7.4 in phosphate buffer, maxima were observed at wave lengths of 225 mμ (log ε 4.334), 258 mμ (log ε 3.823), and 360 mμ (log ε 3.632); minima appeared at 248 and 285 mμ.

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Benzoylvinyl Quaternary Ammonium Salts

BY CHESTER J. CAVALLITO[1]

RECEIVED APRIL 8, 1955

Acetylenes, RC≡CH, react with tertiary amine salts to yield vinyl quaternary ammonium salts, $RCH{=}CHN(R')_3X$.[2] It has been found that phenylethynyl ketone reacts readily with tertiary amine salts to form benzoylvinyl quaternary ammonium salts I in good yield. These compounds might be considered as vinylogs of tertiary amine salts of acid chlorides II.

$C_6H_5COCH{=}CH{-}NR_3X$ (I) $\qquad$ $RCO{-}NR_3X$ (II)

Derivatives of I were prepared from pyridine hydrochloride and from trimethylamine hydrochloride, as white crystalline salts, the former derivative being rather unstable upon standing for several weeks.

Experimental

To a solution of 9.6 g. (0.1 mole) of trimethylamine hydrochloride in 50 ml. of 95% ethanol was added enough trimethylamine to make the solution alkaline. With cooling and shaking, 13 g. (0.1 mole) of phenyl ethynyl ketone[3] was added. The solution was kept at 25 to 30° and, within a few minutes, crystals began to separate. After 30 minutes, the solution was diluted with an equal volume of ether to complete precipitation of the 2-benzoylvinyltrimethylammonium chloride. The product was recrystallized from ethanol. The yield was 88% of the white crystalline salt, cor. m.p. 159° dec.

Anal. Calcd. for $C_{12}H_{16}ONCl$: C, 63.85; H, 7.15; Cl, 15.70. Found: C, 64.16; H, 6.87; Cl, 15.57.

The pyridinium analog was prepared in a similar manner. Two ml. of pyridine base was added to 0.1 mole of pyridine hydrochloride and 0.1 mole of phenyl ethynyl ketone in alcohol. After keeping the mixture at 25 to 30° for 30 minutes (no precipitate formed) an equal volume of ether was added and the oil which separated crystallized upon cooling. The product was recrystallized from dioxane by addition of ethyl acetate or ether; 87%, cor. m.p. 176° dec.

Anal. Calcd. for $C_{14}H_{12}ONCl$: N, 5.70; Cl, 14.43. Found: N, 5.71; Cl, 14.25.

Addition of alkali to aqueous solutions of these quaternaries leads to rapid liberation of the tertiary amine, R_3N. Ultraviolet absorption spectra measurements of the trimethylammonium salt in water show a maximum of ϵ_{288} 7,700 and a minimum of ϵ_{242} 3,200, with no change apparent

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after 24 hr. in solution. In 0.01 *N* sodium hydroxide solution, the maximum is at λ318 with ε 10,300 initially, 8,360 after 24 hr. and 6,800 after 48 hr.[4]

(4) The ultraviolet absorption measurements were supplied by Dr. F. C. Nachod.

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A Study of the Accuracy Obtained in Van Slyke Combustion and Radioassay of Carbon-14 Compounds[1]

BY CLAIR J. COLLINS AND GUS A. ROPP

RECEIVED DECEMBER 3, 1954

Among the more rapid methods of measuring the specific activities of carbon-14 compounds is the procedure in constant use at Oak Ridge National Laboratory. This procedure involves wet combustion of a weighed semi-micro sample of the pure organic compound with Van Slyke mixture and radioassay of the resulting carbon-14 dioxide in a stainless steel ionization chamber.[2]

This method[2] with certain added refinements[3,4] which are necessary when the organic compound to be assayed contains interfering elements such as halogen and nitrogen, can be shown to be capable of giving very precise assays. Since doubt has sometimes been expressed that carbon-14 assays can be obtained with sufficient accuracy for use in isotope effect studies, it is the purpose of the present paper to demonstrate that with carefully controlled wet combustion and radioassay with the vibrating reed electrometer the assays are sufficiently accurate for evaluating isotope fractionation in reactions of carbon-14 labeled compounds. Essentially the problem becomes one of demonstrating that the relative specific activity of a carbon-14 containing compound can be measured within approximately ±0.5% or better as compared with some standard.

In an earlier paper[3] one of the present authors has shown that certain carboxyl-labeled organic acids can be radioassayed with a precision of ±0.5% or better. However, this study involved precision only and no reference was intended to the accuracy[5] of the assay method discussed. If accuracy of an assay method is taken to mean the degree of conformity to the "true" value, then it is apparent that the "true" value must be established in order for accuracy to be calculated. In the present work an arbitrary estimate of the "true" value has been taken as the mean obtained by radioassaying a series of different organic compounds which should have the same molar activity because they were all synthesized from one starting material by processes which involved no isotopic dilution. The reason for this arbitrary choice of a "true" value was that the various members of the reaction series were purified by different methods. Hence, agreement of molar activity among these compounds strongly suggests that they are essentially pure and that the oxidations were essentially quantitative. Variations in molar activities due to the occurrence of isotope fractionations in the reaction series should be immeasurably small for two reasons: (a) most of the reactions are at centers removed from the labeled positions and (b) since the labels are distributed uniformly over six atoms in an aromatic ring, any isotope effects in

CHART I

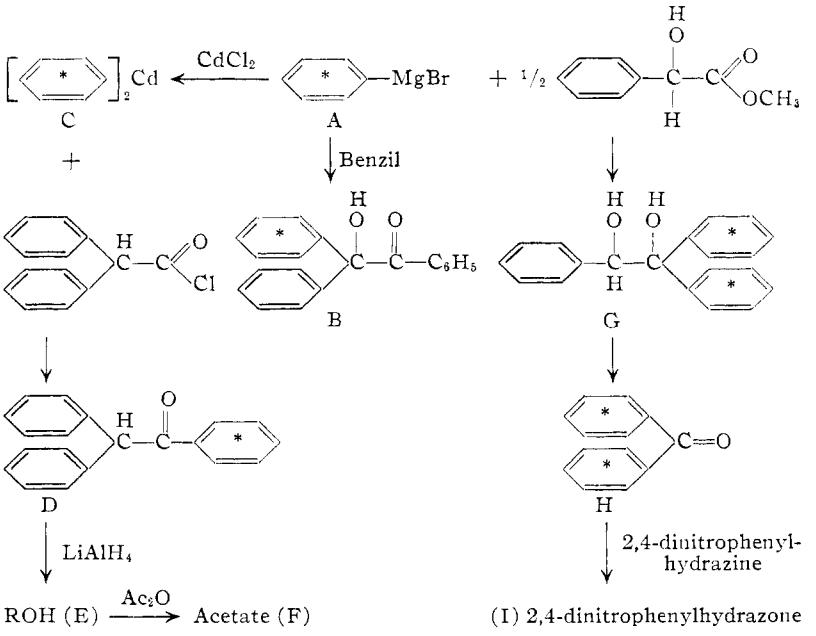

TABLE I

Letter	Compound labeled with carbon-14 in ring	$10^3 \times$ activity values, μc. per mmole	Mean value	Spread, %
A	Phenylmagnesium bromide	Not detd.	..	
B	Triphenylketol	992	992	
C	Diphenylcadmium	Not detd.	..	
D	Benzoyldiphenylmethane	992, 996	994 ± 2	±0.2
E	1,2,2-Triphenylethanol	992	992	
F	1,2,2-Triphenylethyl acetate	985	985	
G	Triphenylethylene glycol[a]	992, 985	988.5 ± 3.5	±0.35
H	Benzophenone	Not detd.	..	
I	Benzophenone 2,4-dinitrophenylhydrazone[a]	996, 982	989 ± 7	±0.7
	Mean and total spread		990.1 ± 5[b]	±0.51
	95% confidence interval		990.1 ± 2.6	±0.26

[a] Molar activity was divided by 2. [b] One value excepted.

(1) This paper is based upon work performed under Contract Number W-7405-eng-26 for the Atomic Energy Commission at Oak Ridge National Laboratory.

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(5) The absolute activity level is, of course, unknown for the compounds referred to in this paper. Since this study is concerned primarily with assays for tracer studies, a method of assay is considered accurate if the measured relative activities of a series of *different* compounds are the same as the relative activities calculated from molar relationships. Precision, however, merely refers to the extent of agreement of a number of assays of the *same* compound.