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]

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[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]

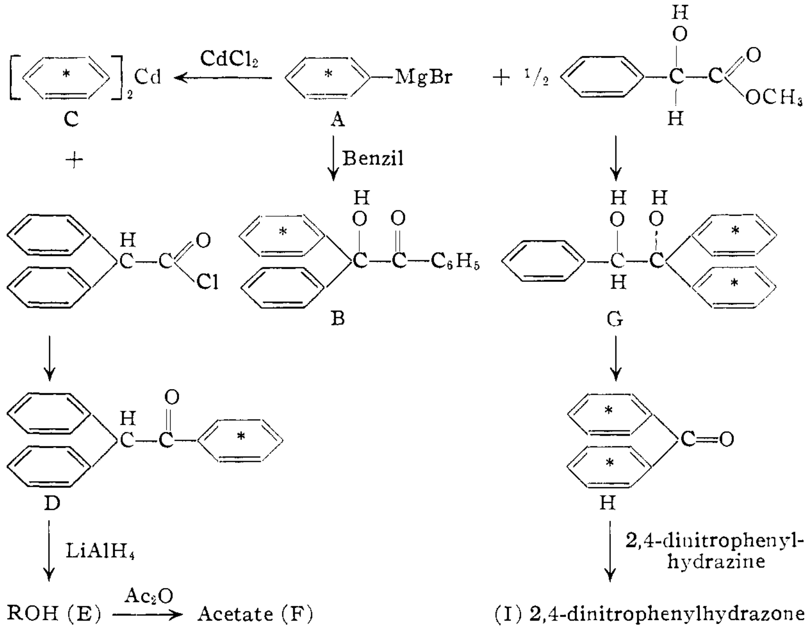
BY CLAIR J. COLLINS AND GUS A. ROPP

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.

CHART I



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

TABLE I

Letter	Compound labeled with carbon-14 in ring	10^3 × 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.

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[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.

[2] O. K. Neville, THIS JOURNAL, **70**, 3501 (1948).

[3] V. F. Raaen and Gus A. Ropp, *Anal. Chem.*, **25**, 174 (1953).

[4] W. A. Bonner and C. J. Collins, THIS JOURNAL, **75**, 3994 (1953).

[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.

reactions affecting the rings probably could not be detected.

In Chart I are shown the reactions by which the compounds of the series were prepared.[6] Table I gives measured molar activities of the various members of the series. Because of the stoichiometry of the reactions some of the molar activities differ by small integral factors. The data of Table I demonstrate that for the series of compounds the agreement with the mean value is about ± 0.5%.

(6) See C. J. Collins and W. A. Bonner, This Journal, **77**, 92 (1955): also C. J. Collins, unpublished work.

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Acetylation of Monocarboxylic Acids with Ketene

BY R. E. DUNBAR AND FLOYD C. GARVEN

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The reaction of ketene with both aliphatic and aromatic acids as a means of preparing mixed anhydrides was first reported by Hurd and Dull[1]

$$CH_2{=}C{=}O + R{-}COOH \longrightarrow R{-}CO{-}O{-}CO{-}CH_3$$

Since this reaction usually occurs at room temperature, in the presence of inert solvents, without the aid of catalysts, it affords a convenient means of preparing and studying other anhydrides. Likewise, since inert solvents are permissible it affords a practical method of acetylating even solid organic acids.

The ketene used was generated by the pyrolysis of acetone using the lamp previously described by Dunbar and Bolstad.[2]

Thirty organic aliphatic and aromatic acids were each individually placed in the gas absorption apparatus designed by Dunbar and Bolstad.[3]

Each acid was then individually treated with ketene until a theoretical excess had been added. Dry ethyl ether was used as a solvent for the solid acids. The mixed acid anhydrides were purified by vacuum distillation or recrystallization from ligroin. Eight such mixed anhydrides, previously prepared with ketene, again were produced and formerly reported constants checked or new constants recorded. Twenty-two additional mixed anhydrides were prepared and their physical constants recorded. Of this group, nineteen have never been reported previously and the remaining three have never been produced previously by the use of ketene. These compounds and constants are recorded in Table I. Concordant elementary analyses for all new compounds are tabulated in Table II.

It has been reported previously,[1] and again observed, that when the size of the two alkyl or aryl groups are decidedly different, the mixed anhydrides showed a decided tendency to disproportionate into the two corresponding simple anhydrides

$$2R{-}CO{-}O{-}CO{-}R' \longrightarrow R{-}CO{-}O{-}CO{-}R + R'{-}CO{-}O{-}CO{-}R'$$

This behavior greatly complicated the purification and determination of physical constants, and frequently required the evaluation of these anhydrides immediately after preparation. As a result of this behavior, many of the constants of these mixed anhydrides have been reported variously from time to time. Fortunately, Whitford[4] has devised a satisfactory method of evaluating the purity of a mixed anhydride, and this test when applied to the freshly prepared anhydrides gave satisfactory confirmation of purity. Furthermore, pure mixed anhydrides show little reaction with sodium carbonate solution,[5] while an equal molecular mixture of two different anhydrides reacts readily. These three tests, as well as the melting points of the anilide, confirm the conclusion that the freshly prepared

TABLE I

MIXED ACETIC ANHYDRIDES, $RCO{-}O{-}COCH_3$

Mixed acetic anhydride	M.p. or b.p. (mm.), °C.	$n^{20}D$ (t, °C.)	d^{20}_4 (t, °C.)
Propionic[a]	31–32 (5)	1.3963	1.013
Butyric[a]	41–43 (5)	1.405	1.00
Iso-butyric[a]	35.7–37 (5)	1.400	0.994
Valeric[a]	55–57 (6)	1.4100	.988
Iso-valeric[a]	48–60 (6)	1.4053	.982
Hexanoic[a]	56–72 (10)	1.401	.955
Benzoic[a]	.....	1.4955	1.501
Stearic[a]	54	1.427 (70)	0.885 (70)
Formic[b]	27–28 (10)	1.388	1.120
Heptanoic[c]	.....	1.414	0.943
Octanoic[c]	.....	1.421	.940
Nonanoic[c]	.....	1.422	.935
Decanoic[c]	.....	1.428	.932
Lauric[c]	.....	1.433	.914
Myristic[c]	46–47	1.432 (35)	.899 (35)
Palmitic[b]	49–51	1.432 (60)	.869 (60)
Crotonic[c]	.....	1.438	1.003
10-Undecenoic[c]	.....	1.436	0.933 (27)
Phenylacetic[c]	.....	1.496 (25)	1.090 (25)
Diphenylacetic[c]	.....	1.524	1.10
Cyclohexylacetic[c]	.....	1.444 (26)	1.019
3-Cyclohexylpropionic[c]	.....	1.449 (22)	1.01 (22)
4-Cyclohexylbutyric[c]	.....	1.450 (22)	0.994 (22)
6-Cyclohexylhexanoic[c]	.....	1.454	0.976
Cinnamic[c]	120–125	...	...
α-Naphthoic[b]	.....	1.568	1.151
o-Toluic[c]	.....	1.513	1.125
m-Toluic[c]	.....	1.504	1.102
p-Toluic[c]	.....	1.516	1 118
2,3-Dichlorophenoxyacetic[c]	.....	1.513	1.308

[a] Previously prepared with ketene but limited properties reported. [b] Previously prepared but not by the use of ketene. [c] New additional mixed anhydrides.

TABLE II

ELEMENTARY ANALYSES OF MIXED ANHYDRIDES

Mixed acetic anhydride	Carbon, % Found	Carbon, % Calcd.	Hydrogen, % Found	Hydrogen, % Calcd.
Heptanoic	62.36	62.76	9.36	9.41
Octanoic	64.34	64.49	10.04	9.74
Decanoic	65.93	67.25	10.26	10.35
Lauric	70.20	69.38	10.91	10.82
Myristic	70.46	71.06	11.27	11.18
Crotonic	55.17	56.24	6.42	6.29
10-Undecenoic	68.80	68.99	10.20	9.80
Phenylacetic	66.70	67.40	6.26	5.66
Diphenylacetic	75.48	75.57	5.95	5.55
Cyclohexylacetic	64.63	65.19	8.99	8.76
3-Cyclohexylpropionic	67.38	66.63	9.45	9.15
4-Cyclohexylbutyric	67.60	67.89	9.63	9.50
6-Cyclohexylhexanoic	70.08	69.96	10.31	10.06
Cinnamic	77.29	77.44	5.42	5.05
o-Toluic	67.79	67.40	5.90	5.66
m-Toluic	67.70	67.40	6.88	5.66
p-Toluic	67.47	67.40	6.03	5.66
2,3-Dichlorophenoxyacetic	45.65	45.65	4.18	3.06

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(1) C. D. Hurd and M. F. Dull, This Journal, **54**, 3427 (1932).

(2) R. E. Dunbar and L. L. Bolstad, *J. Org. Chem.*, **9**, 219 (1944).

(3) R. E. Dunbar and A. N. Bolstad, *Anal. Chem.*, **18**, 337 (1946).

(4) E. L. Whitford, This Journal, **47**, 2934 (1925),

(5) A. Behal, *J. Chem. Soc.*, **78**, 8 (1900).