[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY]

The Chemical Consequences of the $N^{14}(n,p)C^{14}$ Reaction in the Acetamide System and the Implications of Nuclear Recoil as a Tool for Synthesis[1]

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RECEIVED FEBRUARY 18, 1957

Acetic acid-1,2-C_1^{14} and propionic acid-1,2,3-C_1^{14} have been prepared from acetamide irradiated in a nuclear reactor. The distribution of activity in acetic acid averages 62% in the carbonyl carbon and 38% in the methyl carbon. For propionic acid obtained by basic hydrolysis of the amide we found 24% in the carbonyl carbon, 24% in the methylene carbon and 52% in the methyl carbon. These results are interpreted in terms of chemical mechanisms being the determining factors in the labeling process. The formation of acetone-1,2-C_1^{14} with 80% of the activity in the methyl groups and 20% of the activity in the carbonyl group is also reported. Acetonitrile as a product of the radiation chemistry of the system is mentioned. The implication of this work with regard to using nuclear recoil as a tool for the synthesis of labeled compounds is discussed.

The consequences of nuclear recoil in organic nitrogen-containing systems irradiated with neutrons have been the subject of an increasing number of studies.[2] The carbon-14 as it is born receives a recoil energy of about 40,000 e.v. This kinetic energy imparted to the carbon atom causes it to break its chemical bonds and to move into its surroundings. Energy loss occurs by coulomb scattering, collision with other atoms and other processes. When the carbon-14 has been slowed sufficiently to be able to react with its environment, it finds itself a considerable distance from its birthplace,[3-5] the estimates varying from 10 to 3000 Å. The fragmentation and recombination process caused by the recoil atom results in a complex mixture of products. While only one labeled product can result from each event, the amount of energy available for chemical reaction at the site of entrapment of the carbon-14 allows a large variety of compounds to be formed. This is reflected in the diversity of products obtained in an irradiation.

Numerous approaches to this problem are possible. A complete product analysis may conceivably be done. Investigation of specific products and the degree of labeling found in them can be carried out. Compounds with structural features of interest can be investigated in terms of what changes can be made to occur on exposures to carbon recoils. External influences on the products, such as temperature and pressure, might yield useful information. The effect of phase on product distribution is also of interest.

Our studies have been directed toward the elucidation of the mechanism of this process, which in turn should provide the necessary information for the intelligent use of the recoil method in labeling organic compounds. A consideration of the factors enumerated should yield this end.

Constancy of specific activity is the basic criterion for the purity of any product obtained by the recoil method. While chemical purity usually is achieved readily, radiochemical purity, in most cases, is obtained with inordinate difficulty. It became increasingly evident from our work on this and other systems that this difficulty was possibly due to *synthesis*[6] products. It must be kept in mind that these *synthesis* products are formed in microgram amounts or less (with one carbon-14 per molecule), distributed in tens of grams of irradiated material. The presence of these compounds, whose chemical behavior is sufficiently similar to the parent compound to make their separation quite difficult, makes purification a challenging task. Their detection and removal from the parent compound can be effected by carrier methods. Substitution of nitrogen or carbon in the parent compound by carbon-14 results in a *re-entry*[7] product. This product is also removed by carrier methods where applicable.

Results

Acetamide was irradiated in the Brookhaven reactor. Our approach has been to center attention on *re-entry* and *synthesis* products and further to center attention on activity distribution in the compounds themselves. Thus, the *re-entry* product, acetamide-1,2-C_1,[14] and the *synthesis* product, propionamide-1,2,3-C_1,[14] were investigated, and the extent of incorporation of carbon-14 in each position was determined. Both propionic acid and propionamide were added as carriers on the assumption that the synthesized, excited three carbon fragment might in some cases collapse to give propionic acid. The reactions are given in equations 1 and 2.

The hydrolysis was carried out both in acid and in base to investigate possible differences in state of the irradiated material. The hydrolysis in base was carried out on two independently irradiated samples. The results of these runs are listed in Tables I and II. Hydrolysis in acid yielded the results listed in Table III.

(1) Research performed under the auspices of the U. S. Atomic Energy Commission.

(2) (a) R. Edwards, A.C.S. Sixth Southwest Regional Meeting, San Antonio, Texas, 1949. (b) G. Giacomello, *La Ricerca Scientifica*, **21**, 1211 (1951). (c) U. Croatto, G. Giacomello and A. G. Maddock, *ibid.*, **21**, 1598 (1951). (d) M. Zifferero and I. Masi, *Annali di Chim.*, **44**, 551 (1954). (e) M. Zifferero, *ibid.*, **44**, 555 (1954). (f) G. Giacomello and M. Zifferero, *ibid.*, **44**, 558 (1954). (g) M. Zifferero, *ibid.*, **44**, 563 (1954). (h) M. Zifferero and D. Sordelli, *La Ricerca Scientifica*, **26**, 1194 (1956). (i) A. P. Wolf and R. C. Anderson, THIS JOURNAL, **77**, 1608 (1955). (j) A. P. Wolf, C. S. Redvanly and R. C. Anderson, *Nature*, **176**, 831 (1955). (k) A. P. Wolf, B. Gordon and R. C. Anderson, THIS JOURNAL, **78**, 2657 (1956). (l) A. G. Schrodt and W. F. Libby, *ibid.*, **76**, 3100 (1954). (m) A. G. Schrodt and W. F. Libby, *ibid.*, **78**, 1267 (1956).

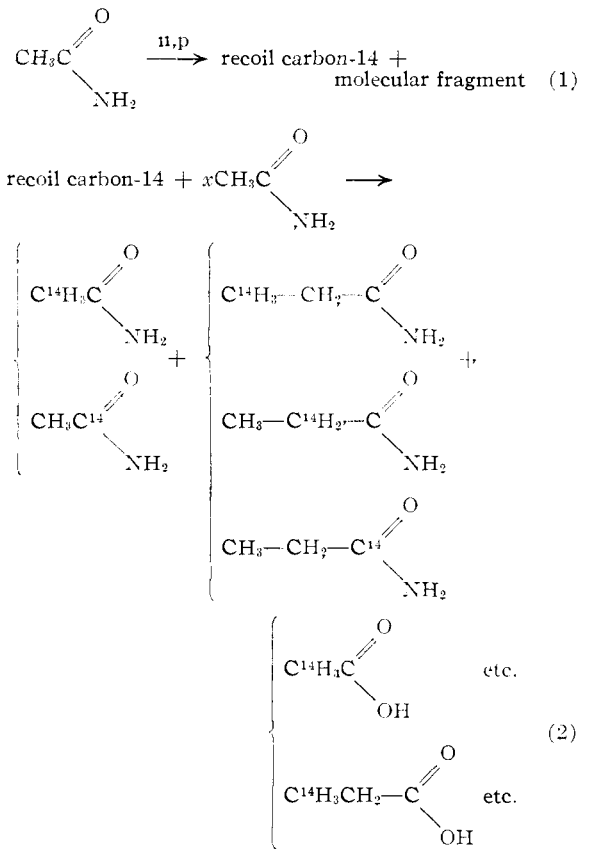
(3) W. F. Libby, *ibid*, **69**, 2523 (1947).

(4) H. Faraggi, *Ann. Phys.*, **6**, 325 (1951).

(5) P. E. Yankwich, *Can. J. Chem.*, **34**, 301 (1956).

(6) By *synthesis* product we mean any product formed which has *one carbon more* than the parent compound, *exclusive* of the carbon analog of the parent compound; *cf.* reference 21.

(7) We prefer *re-entry* to retention in describing this process in order to avoid the implication that the molecule undergoing nuclear transformation is the same molecule which then contains the C^{14} in its skeleton.



While it is clear from these results that synthesis does take place, it becomes evident that the reaction to give "propionamide" involves processes other than a simple replacement of hydrogen.

TABLE I

ACETAMIDE, BASIC HYDROLYSIS, A SERIES

		CH_3—COOH[a]	CH_3—CH_2—COOH[b]			
1.	Specific activity, assay on compd., mμc./mg. C.	0.715		2.18		
2.	Activity relative to total activity in irradiated sample in per cent.	6.44		6.52		
3.	Specific activity, by position, mμc./mg. C.	0.536	0.840	3.30	1.44	1.49
4.	Percentage of summed activity by position	38.9	61.0	52.9	23.1	23.9
5.	Activity accounted for by degradation	96.2		95.4		

[a] Neat. [b] Carrier material.

TABLE II

ACETAMIDE, BASIC HYDROLYSIS, D SERIES

		CH_3—COOH[a]	CH_3—CH_2—COOH[b]			
1.	Specific activity, assay on compd. mμc./mg. C.	0.811		0.886		
2.	Activity relative to total activity in irradiated sample in per cent.	6.83		6.15		
3.	Specific activity by position, mμc./mg. C.	0.608	0.991	1.32	0.646	0.644
4.	Percentage of summed activity by position	38.0	61.9	50.6	24.7	24.6
5.	Activity accounted for by degradation	98.5		98.2		

[a] Neat. [b] Carrier material.

TABLE III

ACETAMIDE, ACIDIC HYDROLYSIS, C SERIES

		CH_3—COOH[a]	CH_3—CH_2—COOH[b]			
1.	Specific activity, assay of compd., mμc./mg. C.	1.24		1.00		
2.	Activity relative to total activity in irradiated sample in per cent.	8.12		4.86		
3.	Specific activity by position, mμc./mg. C.	0.888	1.52	1.46	0.764	0.583
4.	Percentage of summed activity by position	36.8	63.1	52.1	27.1	20.7
5.	Activity accounted for by degradation	97.1		93.7		

[a] Neat. [b] Carrier material.

Discussion

A number of hypotheses have been suggested to account for the results of reactions activated by the n,γ-process. Notable among these are the "billiard ball collision–epithermal collision" hypothesis of Libby[8] and the "random fragmentation–brush heap" hypothesis of Willard.[9]

Yankwich[5] has published a stimulating article on the $N^{14}(n,p)C^{14}$ system in particular. He has provided a rationale for ionic crystal work based mainly on the Seitz and Koehler[10] description of the displacement of atoms during irradiation.

We have considered the formation of labeled species in terms of three steps, each of which may affect product distribution. *The first of these is the slowing down of the carbon-14 and its localization in a reactive site by elastic and inelastic processes. The second step involves the imposition of chemical considerations leading to a variety of intermediate chemical species depending on the structural features of the site and on the energy available for chemical reaction at the entrapment site. The third step involves the collapse of the intermediates to stable compounds or the collapse of reactive fragments, which lead to stable compounds, during the chemical operations carried out on the material subsequent to the irradiation.* Each step involves several factors. The first step is thought of as involving: (1) the energy of the recoil fragment just prior to entrapment in a site, where its motion becomes essentially diffusive in character; (2) the structural features of the fragment during this period and just prior to reaction; (3) the probability of interaction with a particular bond, atom or molecule. The second step is concerned with the chemical considerations which affect the nature of the final product. Involved are: (1) the probability of formation of any of a number of intermediates; (2) the probability that such an intermediate will lead to a stable product; (3) the effect of the surroundings on product distribution. Step three involves essentially three considerations: (1) the probability that a given intermediate will collapse to a compound stable in its surroundings; (2) whether or not the compound will be isolable under the reac-

(8) See reference 3; *cf.* M. S. Fox and W. F. Libby, *J. Chem. Phys.*, **20**, 487 (1952).

(9) "Annual Review of Nuclear Science," Annual Reviews, Inc., Stanford, Cal., 1953, see J. E. Willard, pp. 193–217.

(10) F. Seitz and D. Turnbull, "Solid State Physics," Academic Press, Inc., New York, N. Y., 1956; see F. Seitz and J. S. Koehler, pp. 305–448.

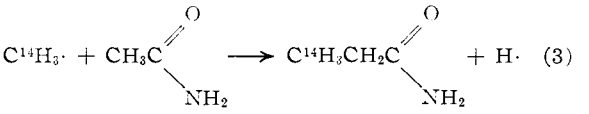
tion conditions; (3) the influence of the isolation method on product distribution.

In theory, a rigorous evaluation of these various factors should allow accurate prediction of product distribution in any given system. The complexity of product distribution from the $N^{14}(n,p)C^{14}$ reaction carried out in relatively simple systems such as ionic crystals has been demonstrated by Yankwich.[11] The greatly increased complexity resulting in an organic system, in which the $Br^{81}(n,\gamma)Br^{82}$ reaction was carried out and in which attention necessarily was centered on the organically bound bromine only, was elegantly investigated by Evans and Willard.[12] These results imply an almost staggering complexity for the product distribution in a system where a recoiling carbon comes to rest in an organic matrix. The complexity of product distribution is certainly evidenced by the work of Schrodt and Libby.[2m]

By centering attention on *re-entry* and *synthesis* products and, further, by centering attention on activity distribution in the compounds themselves, it becomes abundantly clear that chemical forces do indeed play a role in both *re-entry* and *synthesis*. Random distribution is not observed in the *re-entry* product, acetamide-1,2-C_1^{14}, and exclusive hydrogen substitution is not observed in the *synthesis* product, propionamide-1,2,3-C_1^{14}. This is a primary consideration with regard to using the recoil method as a means for preparing labeled compounds. The specificity observed in acetamide probably reflects the effects of factors 1 and 2 in step 2. The higher activity yield of labeled acetic acid from the acid hydrolysis of irradiated material may again reflect these same factors and in addition be a function of factors 1 and 3 of step 3. It may be suggested that the reactive fragment containing carbon-14 will react with its surroundings to give labeled malonic acid. The acid hydrolysis isolation procedure would augment the activity finally assayed as acetic acid since the malonic acid would readily decarboxylate to give acetic acid. It cannot be overemphasized that the nature of the product isolated reflects the structure of the active moiety in the organic matrix only insofar as there is an allowable chemical pathway from the moiety in the matrix to the compound finally isolated. It is not a necessary condition that the active fragment be isolable as a stable chemical entity, nor is it necessary that the final product observed is the result of sequential chemical steps identified with *one* starting moiety.[13]

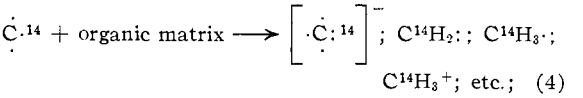
A number of mechanisms can be suggested to account for the distribution of the activity in the propionamide. It is noteworthy that there is *synthesis* and that there is specificity for replacement of a methyl hydrogen. It is, however equally significant that in addition to the 3-position, the 1- and 2-positions are also labeled.

It seems reasonable to assume that the energy associated with the terminal "hot spot" where the recoiling carbon-14 is finally trapped will be of the order of 25–50 e.v.[15,10,14] Clearly then,[15] sufficient energy will be available to allow reaction 3 to occur even though the reaction indicated by equation



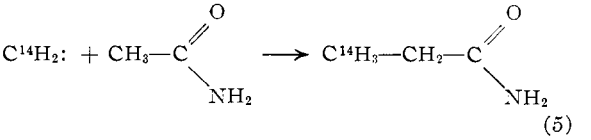
(3)

3 is endothermic in the ordinary sense. This "inversion" reaction is different from that of Hornig, Levey and Willard[16] in that the assemblage after collision need not have sufficient energy to cause bond rupture in the subsequent steps necessary to reduce it to thermal energies. It does not seem likely that such a reaction can take place in the high energy region, since, although the collisions can be between atoms of almost equal mass, the residual energy left, on the average, in such a hot spot will be so high as to allow complete rupture of many bonds, thus resulting in the destruction of any chemical coherence in the center of the spot.[10] The recoiling carbon-14 is an extremely active radical and can react with its surroundings, particularly when its motion becomes essentially diffusive, to give a variety of molecular species such as are indicated in equation 4.



(4)

Doering[17] has shown that methylene produced by the photolysis of diazomethane reacts indiscriminately with the carbon–hydrogen bonds in its surroundings. The reaction given by equation 5 is analogous to Doering's cases, particularly in that a methylene produced from a recoiling carbon can



(5)

certainly have an energy equal to or in considerable excess of the energy of a photolytically produced methylene. Although Doering observed no reaction with carbon–carbon bonds, such a reaction may be possible with a more energetic methylene. Propionamide might therefore be formed by the insinuation of methylene between the two carbons

(11) (a) P. E. Yankwich and J. D. Vaughan, THIS JOURNAL, **76**, 5851 (1954); (b) P. E. Yankwich and W. R. Cornman, Jr., *ibid.*, **77**, 2096 (1955); (c) **78**, 2908 (1956).

(12) J. B. Evans and J. E. Willard, *ibid.*, **78**, 2908 (1956).

(13) A. G. Maddock and N. Sutin, *Trans. Faraday Soc.*, **51**, no. 2, 184 (1955), discuss the effect of the isolation technique on the distribution of activities obtained by neutron irradiation of triphenylarsine and riphenylstibine.

(14) "Reports on Progress in Physics," The Physical Society, London, England, 1955; see G. H. Kinchin and R. S. Pease, pp. 1–51.

(15) The maximum energy transmittable for a collision between two entities is given by

$$E_M = \frac{4M_1M_2}{(M_1 + M_2)^2} E_I$$

where E_I is energy of the incident particle of mass M_1. M_2 is the mass of the struck entity. On the average, the energy transmitted equals $\frac{1}{2}E_M$. The maximum fraction of the kinetic energy of the struck entity which is then available as internal energy is given by

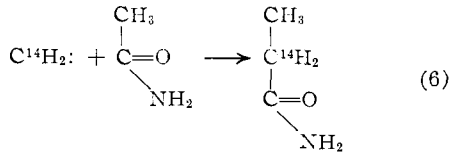
$$E_{Int} = \frac{M_2}{M_1 + M_2} E_T$$

where E_T is the energy transmitted.

(16) J. F. Hornig, G. Levey and J. E. Willard, *J. Chem. Phys.*, **30**, 1556 (1952).

(17) W. von E. Doering, R. G. Buttery, R. G. Laughlin and N. Chaudhuri, THIS JOURNAL, **78**, 3224 (1956).

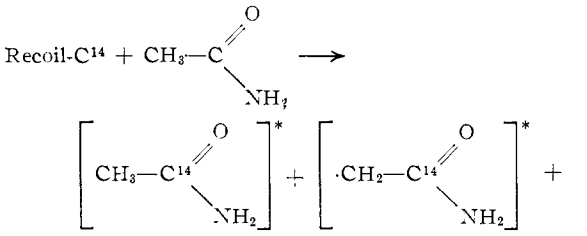
of acetamide as given in equation 6. The formation of cyclopropanone, which is known to open to propionic acid, might also account for some of the labeled acid.



(6)

Activity in the carbonyl group might be the result of the formation of an intermediate which can then undergo skeletal rearrangement and shifting of the oxygen. An intermediate which becomes stripped of oxygen and is then reoxidized is also a possibility.

Here as with acetamide, the formation of an intermediate which subsequent chemistry can transform to propionic acid is a possibility. The case of carbonyl labeling is indicated by equations 7 and 8.



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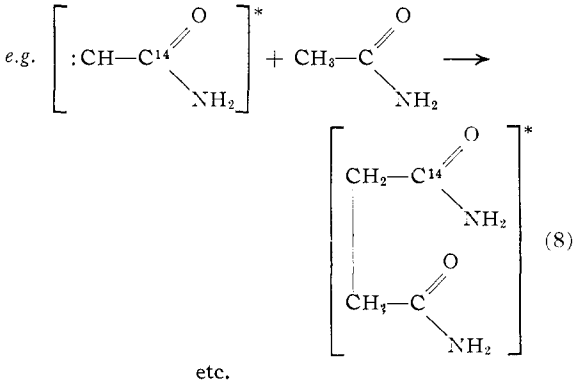
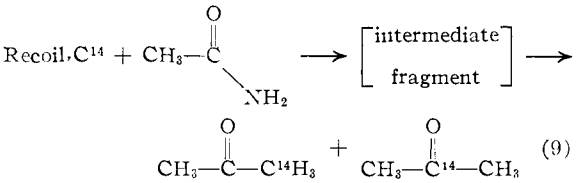


(8)

There is some indication that succinic acid may decarboxylate to give small amounts of propionic acid when in the presence of base and traces of heavy metal salts. Alternatively the excited succinamide might break a methylene to carboxamide bond to give a radical which would then abstract a hydrogen from its surroundings.

Experiments which will distinguish between these various mechanisms are in progress at the present time.

Acetone-1,2-C_1^{14}.—In our work on the acridine–anthracene[2] system it was shown that the ratio of activity as acridine to activity as anthracene averaged 18/1. A completely random *re-entry* would result in a ratio of 13/1. Muxart[18] has obtained an average ratio of 19/1 in the 5,6-benzacridine–5,6-benzanthracene system where the statistical ratio is 17/1. The activity as acetone in the carrier material was found to be 0.13% of the total activity produced. A ratio of 49/1 was found, whereas the statistical ratio for acetone is 2/1. The reaction is given by equation 9.



(9)

The value of these ratios in the mechanistic sense probably has real meaning when the molecules formed by *re-entry* do not have fundamentally different chemical stabilities. We also need to assume that the excited *re-entered* molecules will behave similarly in their surroundings in the chemical sense. For example, the excited states of acridine and anthracene are sufficiently similar that we might expect the *re-entered* acridine and the active anthracene from nitrogen replacement to have roughly equal probabilities of being reduced intact to thermal energies. Acridine which might be lost by the formation of biacridine might be mirrored by bianthracene formation from anthracene. Once formed, the chemical stabilities and their stability to radiation damage are nearly equal in terms of total product destroyed. This parallel behavior then lends validity to the conclusions one can draw from the statistical nature of the replacement.

The applicability of these hypotheses is supported by the results with acetone. Here the intermediates leading to acetone and *re-entered* acetamide probably bear no relationship. Further the radiation stabilities of these two materials, once formed, are markedly different. The deviation from statistical behavior is then a reflection of the chemistry of the system after *re-entry* has occurred, emphasizing the fact that a purely physical picture of the chemical results from a system containing recoil fragments is not sufficient.

In addition, the degree of labeling by position in acetone, being 20% in the carbonyl carbon and 80% in the indistinguishable methyl carbons, is significant in that it necessitates a mechanism involving not only *re-entry* but also rearrangement of the species after *re-entry*.

Acetonitrile.—The rate of energy absorption by the system due to the recoiling carbons is about 2.7×10^{15} e.v./sec./gram of acetamide and is about 3.5×10^{16} e.v./sec./gram of acetamide for the ejected protons. A crude calculation based on the "Dosimetry of Reactor Radiations" work by Richardson, Allen and Boyle[19] indicates that about 1.2×10^{17} e.v./sec./gram of acetamide are being dissipated in the sample due to pile irradiation. Since only 1.7% of the energy absorbed by the sample can be attributed to the recoil fragment,[20]

(18) R. Muxart, *Compt. rend.*, **242**, 2457 (1956).

(19) "Proceedings of the International Conference on the Peaceful Uses of Atomic Energy," Vol. XIV, United Nations, New York, N. Y., 1956; see D. M. Richardson, A. O. Allen and J. W. Boyle, pp. 209–212.

(20) Additional reactions such as $C^{13}(n,\gamma)C^{14}$ play an insignificant part in energy deposition.

it seems unlikely that acetonitrile formation is a specific effect caused by the knockons from carbon-14. It is, however, interesting to speculate whether or not acetonitrile is a specific product of knockons by fast neutrons, ejected protons, and by recoil fragments or whether it is formed by means of the gross radiation the sample receives.[21]

Use of Nuclear Recoil as a Method for Labeling Organic Compounds.—Several factors must be evaluated in using nuclear recoil as a labeling method. The level of activity desired in the compound, the degree of labeling by position and the ease with which the labeled compound may be prepared by conventional methods must be considered.

It is necessary first to decide on the source of the required nitrogen. In many of the cases reported, the compound serves as its own nitrogen source. If the compound to be labeled contains no nitrogen, it may be mixed with a nitrogen source which will form a homogeneous solution[22] with the compound. This source must provide a high nitrogen to carbon ratio, be radiation stable and be easily removable from the desired compound. Derivatization of the compound with nitrogen-containing compound is a third possibility.

The optimum irradiation time is based on minimization of radiation damage such that a sensible amount of chemically pure material can be recovered. This must be coupled with maximization of activity produced. A review by Collinson and Swallow[23] on the radiation chemistry of organic substances can serve as a guide for the qualitative evaluation of the expected radiation damage. For example, acridine,[21] after 20 days of pile irradiation, is 76% recoverable, whereas L-alanine[24] is only 22% recoverable after a six-day irradiation.

Calculation of the total activity produced during the irradiation can be carried out easily.[21] The fraction of the total activity found in the *re-entry* product depends strongly on the nature of the compound to be *re-entered*. We have observed a wide range of values: *e.g.*, 0.2% in alanine,[24] $\sim$ 1% in ethylpyridine,[24] 1.5–2% in benzene[2j,k] (not corrected for dilution by the nitrogen source), 3.5% in acridine, to 8.0% in acetic acid from the acid hydrolysis of acetamide. These percentages are corrected for the material lost by radiation damage. Higher *re-entry* figures have been reported for some organic compounds, but they do not serve as a basis for calculation in the absence of rigorous proof of constancy of the specific activities observed. The specific activity obtainable is then given by equation 10.

$$S.A._x = \frac{A_T \times P_T}{M_C \times 100} \qquad (10)$$

$S.A._x$ = estimated specific activity expected in compound x in activity per milligram of carbon
A_T[25] = total activity produced during irradiation
P_T[26] = percentage of the total activity produced expected to be found in compound x
M_C = milligrams of carbon in total sample of compound x

This calculation assumes no radiation damage in evaluating M_C and thereby gives a common basis for comparison between compounds. Clearly the specific activity is the same for any compound derived from the compound irradiated provided the derived compound has the same number of carbons per mole and provided no carbons were exchanged.

The actual chemical yield of *re-entry* product is dependent on the amount not destroyed by radiation damage. After the material is shown to be chemically and radiochemically pure, it can be diluted as desired, depending on the detection limits of the assay method used. With our present assay equipment the acetic acid produced from the acid hydrolysis of acetamide (1.24 mμc./mg. C) could have been diluted 500-fold without appreciably changing[27] the precision of assay.

It must be remebered that the degree of labeling in each position *is not* uniform throughout the molecule. This must be taken into account in any further use to which the labeled compound is to be put.

The *re-entry* product resulting from replacement of nitrogen in the original compound, provided it acts as its own nitrogen source, and all *synthesis* products present a different picture. In theory, compounds with extremely high specific activity could be produced in this way. If it were experimentally feasible to isolate the anthracene-C_1^{14} produced from acridine,[28] we would have a compound with one carbon-14 in each anthracene molecule. It is, however, practical to adjust the specific activity desired by adding a greater or lesser amount of carrier at the end of the irradiation. Again the percentage of the total activity to be expected in the N-replacement and *re-entry* and/or *synthesis* products is estimated and sufficient carrier added to give the desired specific activity. For example, the specific activity of the propionic acid in the experiments reported here could have been increased by a factor of ten in any series by a corresponding decrease in the amount of added carrier. By the choice of a proper system and a high N/C

(21) Production by knockons would necessarily proceed with very high efficiency since an estimate of the energy deposited by "knockons" (total) alone indicates it to be a borderline possibility at best. This question might be resolved by comparing acetonitrile production from pile irradiation (at maximum fast neutron flux) with acetonitrile production from irradiation in a cobalt-60 source under conditions of identical total energy deposition.

(22) In labeling benzene,[2k] a mixture of benzene and 2-methylpyrazine was used; *cf.* 2a,l,m. We have used liquid ammonia without notable success because of the difficulty in finding appropriately soluble materials. Heterogeneous mixtures are not suitable because of the comparatively short range of the recoiling carbon-14. The percentage of carbon recoils reaching organic material in a mixture such as finely ground ammonium nitrate and anthracene is too small to make the method feasible.

(23) E. Collinson and A. J. Swallow, *Chem. Revs.*, **56**, 471 (1956).

(24) A. P. Wolf and C. S. Redvanly, unpublished work.

(25) While this is the value calculated, it should be noted (*cf.* ref. 33) that the activity produced experimentally is usually higher than the calculated value. Thus the value obtained as SA is a minimum in most cases. In no case that we have observed has the experimental value been greater than $1.8A_T$. An average of our published and unpublished work is $1.3A_T$. This variation from theoretical may be due to the primitive technology involved in carrying out these irradiations. An accurate value for the slow neutron flux integrated over the whole irradiation is not as yet available.

(26) As indicated in the text the value for P_T varies rather widely. A judicious guess based on the published work[2] is required in order to evaluate P_T. We assume a value of 3% in lieu of any other information.

(27) D. R. Christman and A. P. Wolf, *Anal. Chem.*, **27**, 1939 (1955).

(28) A twenty day irradiation of 24 grams of acridine at a flux of 3×10^{13} neutrons/cm.^{2} sec. produces about 0.6 microgram of anthracene-C_1^{14} dispersed in the acridine.

ratio, it may be possible to obtain a *synthesis* or N-replacement product carrier free.

As with the *re-entry* product, it must be remembered that the labeling is not specific. In the cases observed to date, the majority of the label is found in the expected position in the N-replacement product, and in the *synthesis* products: methyl group in acetone; methyl group in propionamide; methyl group in toluene.[29] The percentage found in the expected position is not, however, the same in all compounds. Again the application to which the compound is to be put is the determining factor.

The quantities of materials that can be produced are subject to the geometry of the facility available. The water-cooled facility with maximum available flux in the Brookhaven reactor can accommodate about 80 grams (assumed density = 1) of a liquid sample per irradiation. About 100 grams of tightly packed solid can be irradiated in the same facility. Radiation times range from 4 to 30 days.

It is hoped that this outline based on work to date will aid in deciding whether or not the method is applicable to the compound in question. The complexity of the purification is usually the deciding factor. With the advent of vapor phase chromatography, however, labeled liquid alkanes, aromatics, alcohols, acids, etc., can be prepared easily and quickly. Carrier-free materials may also be within reach.[29] It is clear that adequate fractionation techniques hold the key to the application of nuclear recoil as a labeling method.

The elucidation of the mechanisms involved in these reactions is the continuing interest of the authors. The investigation of the effect of structure and phase on *re-entry* and *synthesis* is being pursued.

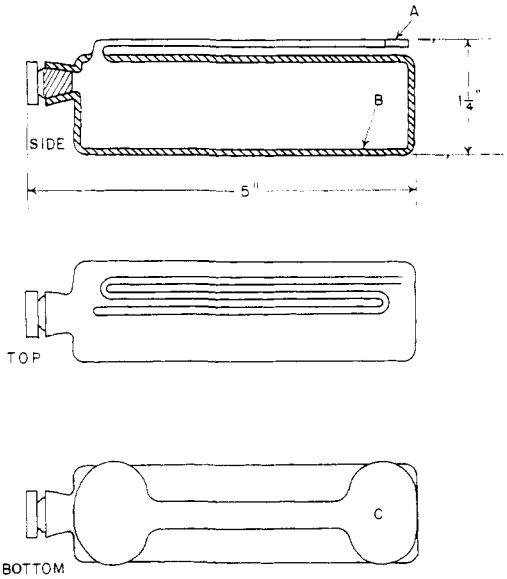


Fig. 1.—A, 0.5 mm. i.d. quartz capillary; B, 25 mm o.d. quartz tubing; C, ends blown flat and then ground for stability in boat.

(29) *Cf.* R. M. Lemmon, F. Mazzetti, F. L. Reynolds and M. Calvin, THIS JOURNAL, **78**, 6414 (1956). This case is closely analogous to the recoil method.

Experimental[30]

Irradiation.—The samples were irradiated in the Brookhaven reactor at an approximate flux of 4×10^{12} neutrons/cm.^{2} sec. The fast neutron flux was $\leqq 10^{12}$ neutrons/cm.^{2} sec. The gamma exposure was $\leqq 2 \times 10^6$ r./hr. In order to correct approximately for fluctuations in neutron density during irradiation, an integrated value for the total irradiation is reported in megawatt hr.[31] All irradiations were carried out in a facility operating at 30°. After irradiation, at least ten days were allowed for the decay of short lived activities in the quartz containers.

Packaging.—The type of container used is pictured in Fig. 1. The plug is held in place with Kronig cement. The capillary aids in reducing the amount of air in continuous contact with the sample, while still allowing the gases formed during the irradiation to escape. Appreciable gas pressures build up on irradiation of many aliphatic compounds. Closed systems are contra-indicated for pile irradiation of these samples. Safe irradiation of samples evolving gases can be accomplished in closed systems if necessary.[32]

Fisher Certified Reagent acetamide, m.p. 80–81°, was used for all irradiations.

Anal. Calcd. for C_2H_5ON: C, 40.7; H, 8.5. Found: C, 41.1; H, 8.4.

Processing of Acetamide-C_1^{14}. Addition of Carriers. A Series.—Two samples of acetamide (1, 21.077 g.; 2, 23.662 g.) received an irradiation of 4965 megawatt hours; acetamide survival 74 ± 4% (average both samples).

Sample 1.—*Anal.* Calcd. for C_2H_5ON: C, 40.7; H, 8.5. Found: C, 39.0 ± 0.9; H, 8.2; 12.06 ± 0.93 mμc./mg. C.[33]

Sample 2.—*Anal.* Found: C, 40.1 ± 1.2; H, 8.4; 10.27 ± 0.51 mμc./mg. C.[33]

Samples one and two were combined before carrier material was added. A weighted average for the carbon value and the carbon-14 activity is given; C, 39.6 ± 1.5; 11.11 ± 1.06 mμc./mg. C. The total activity for the irradiated sample was based on the carbon value before irradiation multiplied by the specific activity value obtained by assay. The total activity produced was 0.202 mc.

Transfer of 35.999 g. of the mixture to a bulb which could be connected to a vacuum line was carried out. Carriers were added to the bulb as follows: (1) 5.117 g. of propionamide, (2) 5.13 g. of acetone, (3) 4.806 g. of propionic acid. The vacuum bulb was capped and raised to a temperature of 70°. Copious gas evolution occurred during the melting and dissolution process. The liquid was resolidified in an ice-bath.

Acetone-1,2-C_1^{14}.—The vacuum bulb containing the resolidified homogenate was evacuated through two liquid N_2 traps connected to a high vacuum system. A vacuum

(30) Microanalyses on inactive samples were performed by F. Schwarzkopf, Woodside 77, N. Y. Radioassays were performed by P. R. Hansell and D. R. Christman of this Laboratory. The method of D. R. Christman, N. E. Day, P. R. Hansell and R. C. Anderson, *Anal. Chem.*, **27**, 1935 (1955), was used. A minimum of two assays were performed on each compound. The standard deviation for any given assay is 1–2% ($\sigma = \sqrt{\text{total counts}} \times 100/\text{total counts}$). The root mean square error encompassing all errors inherent in the analysis is not greater than 5%.

(31) Reference 2i explains the method of calculation used.

(32) A. P. Wolf and A. Oltmann, Brookhaven National Laboratory Report No. 2811.

(33) The deviations given here are average deviations calcd. from the experimental data. Data was based on 9 determinations for sample 1 and 7 determinations for sample 2. It has been our observation on all but the most radiation-stable compounds that precise C, H data and precise radioassays are difficult to obtain on the unprocessed pile material. Various factors such as inhomogeneity of the sample and the presence of other radioactive species have been investigated as possible causes for these effects. Negative results have been obtained. In particular the CO_2 from combustions has been purified both by cycling through $BaCO_3$ and by passing the CO_2 through a Vapor Phase Chromatographic (V.P.C.) column. No significant improvement in statistics was noted. The disparity between samples 1 and 2 is due to their location in the pile during the irradiation, the former having been close to the position of maximum flux. It should be noted that the megawatt hour reading serves mainly as an approximate irradiation value.

transfer of the added acetone and other volatile constituents was effected. A negligible amount of the propionic acid was transferred. An 8.21-g. sample was obtained. The sample was separated into four fractions by distillation. Fractionation in an 8-inch glass helix packed column served to further purify the acetone fraction. Material boiling at 56° was converted to the semicarbazone. The semicarbazone was recrystallized twice from H_2O, m.p. 186°.

Anal. Found: 0.0674 mμc./mg. C., cor. for inactive carbon. The bisulfite addition product was prepared. *Anal.* Found: 0.069 mμc./mg. C.

Iodoform was prepared from the acetone using the procedure described by Meinwald.[34] *Anal.* Found: 0.082 mμc./mg. C.

Acetic Acid-C_1^{14}.—After vacuum transfer of the volatile constituents of the vacuum bulb had been effected, the residue, 40.0 g., was transferred to a r.b. flask. The mixture was hydrolyzed with 50 g. of sodium hydroxide in 200 cc. of H_2O. The solution was taken to *p*H 2 with sulfuric acid. The precipitate formed on neutralization was removed and washed with water. The combined aqueous solutions were continuously extracted with ether. The ether was removed through a Vigreux column. An acid fraction, 35.2 g., obtained on continued heating was used to charge the pot of a 12 mm. i.d. by 380 mm. long, $^3/_{32}$'' glass helix packed column. Mesitylene was used as a chaser.

A center cut of acetic acid was used for all derivative preparations. The cut was contaminated with a trace of mesitylene. *Anal.* Found: 0.721 mμc./mg. C.; b.p. 117–118.5°.

The *p*-toluidide of acetic acid was prepared, m.p. 145.5-146°. *Anal.* Calcd. for $C_9H_{11}ON$: C, 72.5; H, 7.4. Found: C, 72.8; H, 7.6; 5th recrys., 0.155 mμc./mg. C.; 7th recrys, 0.157 mμc./mg. C.; 0.702 mμc./mg. C., cor. for inactive carbon.

Acetic acid was converted to the thallous salt[35] for purification. Carbon dioxide free solutions of thallous hydroxide were prepared from thallous hydroxide obtained from Varlacoid.[36] The acid was carefully titrated to a phenolphthalein end-point. Care had to be taken to eliminate any excess of thallous hydroxide by back titration with an inorganic acid. A slight excess of thallous hydroxide makes subsequent purification more troublesome. After vacuum evaporation of the aqueous solution, the residue was triturated with absolute ethanol. The alcohol solution usually was treated with Darco and again taken to dryness. Thallous acetate can be recrystallized readily from absolute ethanol. It comes out of solution in shining platelets, m.p. 126.5–127.5°. The ease of preparation and purification coupled with the non-hygroscopic nature of this salt makes it ideal as a derivative and as a source of acetic acid in degradation procedures.

Anal. Calcd. for $TlC_2H_3O_2$: C, 9.1; H, 1.2. Found: C, 9.2; H, 1.2; 3rd recrys., 0.723 mμc./mg.C.

Propionic-C_1^{14} Acid.—The carrier propionic acid–propionamide was isolated as propionic acid during the fractionation described under acetic-C_1^{14} acid. The propionic acid fraction contained considerable mesitylene. The *p*-toluidide of propionic acid was prepared, m.p. 123.5–125°.

Anal. Calcd. for $C_{10}H_{13}ON$: C, 73.6; H, 8.0. Found: C, 73.5; H, 8.1; 3rd recrys., 0.675 mμc./mg. C.; 5th recrys., 0.654 mμc./mg. C.; 2.21 mμc./mg. C., cor. for inactive carbon.

Thallous propionate was prepared, m.p. 190–190.5°.[37] *Anal.* Calcd. for $TlC_3H_5O_2$: C, 13.0; H, 1.8. Found: C, 13.1; H, 1.8; 2.20 mμc./mg. C.

A further fractionation of 6 g. of this material was carried out in a 50-plate concentric tube column. Propionic acid boiling at 141.2° was used for analysis and degradation. A small trace of mesitylene still contaminated the sample.

Anal. Found: 2.13 mμc./mg. C. (cor. for trace of mesitylene).

Degradation of Acetic Acid-1,2-C_1^{14}.—The method used was an adaptation of the method of Phares[38] based on the Schmidt reaction. *Anal.* Found: CO_2, 0.840 mμc./mg. C. (2 runs).

The methylamine produced was converted to N-phenyl-N'-methylthiourea, m.p. 113–113.5°. *Anal.* Calcd. for $C_8H_{10}N_2S$: C, 57.8; H, 6.1. Found: C, 57.8; H, 6.1; 0.536 mμc./mg. C., cor. for inactive carbon.

Degradation of Propionic Acid-1,2,3-C_1^{14}.[39]—*Anal.* Found: CO_2, 1.49 mμc./mg. C. (4 runs).

The ethylamine produced from one of the runs was converted to N-phenyl-N'-ethylthiourea. *Anal.* Calcd. for $C_9H_{12}N_2S$: C, 60.0; H, 6.7. Found: C, 60.2; H, 6.7; 2.39 mμc./mg. C., cor. for inactive carbon (1 run).

The ethylamine was also converted to acetic acid by the method of Phares and degraded further. *Anal.* Found: CO_2, 1.44 mμc./mg. C. (2 runs).

The methylamine produced was converted to N-phenyl-N'-methylthiourea. *Anal.* Found: 3.30 mμc./mg. C. corr. for inactive carbon (3 runs).

Acetonitrile.—A higher boiling fraction obtained from the fractionation of the carrier acetone sample was shown to be infrared spectroscopically identical with authentic acetonitrile.

Processing of Acetamide-C_1^{14}; Addition of Carriers. C Series.—A sample of acetamide (32.745 g.) received an irradiation of 6254 megawatt hours. Acetamide survival 46% (estimated). *Anal.* Found: 15.27 ± 0.25 mμc./mg. C.[40] Total activity produced, 0.203 mc. The carriers (9.928 g. of propionamide, 9.663 g. of propionic acid) were added directly to the quartz irradiation vessel containing the remaining acetamide (31.825 g.). The contents of the vessel were homogenized by melting.

Acetic Acid-C_1^{14}.—The contents of the quartz vessel were transferred (48.768 g.) to a round-bottom flask containing 200 g. of sulfuric acid and 200 cc. of water. After boiling for 16 hr. the solution was distilled. The distillate was treated with 0.88 g. of potassium permanganate to remove the sulfite ion present. The solution was continuously extracted with ether for 24 hr. Removal of the ether left 59.53 g. of an acid–water mixture. A center cut, b.p. 118–118.5°, obtained by fractionation as previously described under Acetic acid-1,2-C_1^{14}, A series (no mesitylene chaser was used), was used for all derivative preparations. *Anal.* Found: 1.22 mμc./mg. C.

The *p*-bromophenacylacetate was prepared, m.p. 83–84.5°. *Anal.* Found: 1.26 mμc./mg. C., cor. for inactive carbon.

Thallous acetate was prepared as previously described. *Anal.* Found: 5th recrys., 1.23 mμc./mg. C., 7th recrys., 1.23 mμc./mg. C.

Acetic acid from this cut was purified by vapor phase chromatography (V.P.C.) on a 1.2 cm. × 250 cm. column containing crushed firebrick, Johns–Manville C-22, 80–100 mesh; 50% by weight of 90% Dow Corning (D.C.) 710 plus 10% stearic acid constituted the liquid phase. The column temperature was 120°. The heart cut of the acetic acid fraction was converted to the thallous salt. The dissolved salt was treated with Darco and subsequently recrystallized. *Anal.* Found: 1.24 mμc./mg. C.

Propionic Acid-C_1^{14}.—Propionic acid from the fractionation described under acetic acid-C_1^{14} was analyzed. *Anal.* Found: 1.02 mμc./mg. C.

Thallous propionate was prepared as previously described. *Anal.* Found: 1.00 mμc./mg. C.

Propionic acid was converted to methyl propionate by treatment with diazomethane. The ester was purified by V.P.C. on a column such as described under acetic acid-C_1^{14}. The liquid phase was Dow–Corning 710. After hydrolysis of the ester fraction with base, the solution was acidified and then extracted with ether. The thallous salt was prepared from the ether solution. Analysis of the salt followed its treatment with Darco and subsequent recrystallization. *Anal.* Found: 0.982 mμc./mg. C.

Degradation of Acetic Acid-1,2-C_1^{14}.[38]—*Anal.* Found: CO_2, 1.52 mμc./mg. C. (3 runs).

The methylamine produced was assayed as N-phenyl-

(34) J. Meinwald, THIS JOURNAL, **77**, 1617 (1955).

(35) R. Walter, *Ber.*, **59**, 962 (1926).

(36) 116 Broad Street, New York 4, N. Y.

(37) *Cf.* preparation of thallous acetate and reference 35.

(38) E. F. Phares, *Arch. Biochem. Biophys.*, **33**, 173 (1951). Our modification will be published in a forthcoming article in *Anal. Chem.* Counting tubes were filled with the CO_2 produced in the Schmidt reaction. The assays were carried out using both the neat acid and the thallous salt as starting material.

(39) *Cf.* degradation of acetic acid-C_1^{14}. The assays were carried out using both the neat acid and the thallous salt as starting material.

(40) Average deviation on experimental data.

N'-methylthiourea. *Anal.* 0.888 mμc./mg. C., cor. for inactive carbon (3 runs).

Degradation of Propionic Acid-1,2,3-C_1^{14}.[39]—*Anal.* Found: CO_2, 0.583 mμc./mg. C. (4 runs).

The ethylamine produced from one of the runs was converted to N-phenyl-N'-ethylthiourea. *Anal.* Found: 1.10 mμc./mg. C., cor. for inactive carbon.

Acetic acid made from the ethylamine was degraded. *Anal.* Found: CO_2, 0.764 mμc./mg. C.

The methylamine produced was assayed as N-phenyl-N'-methylthiourea. *Anal.* Found: 1.46 mμc./mg. C., cor. for inactive carbon.

Processing of Acetamide-C_1^{14}; Addition of Carrier D Series.—A sample of acetamide (37.150 g.) received an irradiation of 5174 megawatt hours; acetamide survival, 65 ± 3%. *Anal.* Found: 11.86 mμc./mg. C. Total activity produced, 0.179 mc. The carriers (9.0128 g. of propionamide, 9.0413 g. of propionic acid) were added to 26.428 g. of irradiated acetamide. The mixture was homogenized by liquefaction.

Acetic Acid-C_1^{14}.—Hydrolysis of the mixture with base, isolation of the acids and fractionation of the acids was carried out as previously described under Acetic acid-C_1^{14}, A series (no mesitylene chaser was used).

Acetic acid, b.p. 118.5–119.5°, was further purified by V.P.C. The column previously described was used. The liquid phase was 90% D.C. 710 plus 10% stearic acid. A heart cut of the acetic acid was taken. This material was converted to thallous acetate and subsequently purified. *Anal.* Found: 0.811 mμc./mg. C.

Propionic Acid-C_1^{14}.—Propionic acid, b.p. 140.5–141.1°, from the fractionation of the acid fraction was purified by V.P.C. as described under acetic acid-1,2-C_1^{14}. The heart cut of this material was converted to thallous propionate and subsequently purified. *Anal.* Found: 0.886 mμc./mg. C.

Degradation of Acetic Acid-1,2-C_1^{14}.—The thallous salt was degraded. *Anal.* Found: CO_2, 0.991 mμc./mg. C.

The methylamine was assayed as N-phenyl-N'-methylthiourea. *Anal.* Found: 0.608 mμc./mg. C., cor. for inactive carbon.

Degradation of Propionic Acid-1,2,3-C_1^{14}.—The thallous salt was degraded. *Anal.* Found: CO_2, 0.644 mμc./mg. C.

The ethylamine was assayed as N-phenyl-N'-ethylthiourea. *Anal.* Found: 0.959 mμc./mg. C., cor. for inactive carbon.

Acetic acid made from the ethylamine was degraded. *Anal.* Found: CO_2, 0.646 mμc./mg. C.

The methylamine produced was assayed as N-phenyl-N'-methylthiourea. *Anal.* Found: 1.32 mμc./mg. C., cor. for inactive carbon.

UPTON, NEW YORK

[CONTRIBUTION FROM THE COLLEGE OF CHEMISTRY AND PHYSICS, THE PENNSYLVANIA STATE UNIVERSITY]

The Rate of Hydration of Methylenecyclobutane and the Effect of Structure on Thermodynamic Properties for the Hydration of Small Ring Olefins[1]

BY PETER RIESZ, ROBERT W. TAFT, JR., AND ROBERT H. BOYD

RECEIVED FEBRUARY 4, 1957

The effects of small ring structure on the position of equilibrium in hydration of olefins to *t*-carbinols have been found to be of substantial magnitude and in the directions predicted by Brown's I-strain rules. The relatively small effects of ring size on the rates of hydration which are found indicate that there is no appreciable covalent bonding of a water molecule to carbon in the reaction transition state. It is concluded from the observed effects of structure on the rate of hydration that the π-complex provides a more suitable model for the molecular structure (nuclear arrangement) of the transition state than does the classical carbonium ion. The transition state exhibits the property of possessing carbonium ion character with respect to electronic, but not molecular, structure. This property is attributed to more sluggish nuclear than electronic rearrangements in attaining the transition state.

The rates of hydration of gaseous methylenecyclobutane and 1-methyl-1-cyclobutene to 1-methylcyclobutanol in aqueous nitric acid solution have been determined by measuring the rate of drop in the saturated vapor pressure of olefin over the solution in which the reaction occurs.[2]

Demjanow and Dojarenko have shown that methylenecyclobutane is converted to 1-methylcyclobutanol by treatment with 66% H_2SO_4.[3] We find no evidence that rearranged products are formed during the hydration.

Rates and equilibria in the hydration of the small ring olefins have been investigated in order to provide critical evidence concerning the reaction mechanism. Taft has proposed that the rate-determining step in the aqueous acid-catalyzed aliphatic olefin–*t*-carbinol interconversion involves the isomerization of π-complex and carbonium ion intermediates.[4] Several criteria of mechanism indicate that the same mechanism is involved for the small ring as for the open chain olefins.[4,5]

By combining the results of the present research with those of previous investigations, we have obtained data on the effect of structure on the thermodynamic equilibrium and rate properties for the hydration of small ring olefins relative to that for corresponding open chain olefins. This information provides new evidence in support of Taft's mechanism.

Experimental

Methylenecyclobutane.—The zinc reduction of pentaerythrityl tetrabromide[6] was carried out in a manner similar to that described by Roberts and Sauer.[7] In order to separate the olefins in the crude product from spiropentane, the reaction product was extracted several times with almost saturated aqueous silver nitrate solution and the olefin was regenerated by warming the solution.[8] The crude olefin product was fractionated through a 40-inch column packed

(1) The work reported herewith was carried out as Project NR055, 295 between the Office of Naval Research and The Pennsylvania State University. Reproduction in whole or in part is permitted for any purpose of the United States Government.

(2) J. B. Levy, R. W. Taft, Jr., D. Aaron and L. P. Hammett, THIS JOURNAL, **73**, 3972 (1951).

(3) I. N. Demjanow and M. Dojarenko, *J. Russ. Phys. Chem. Soc.*, **45**, 176 (1913); *Chem. Zentr.*, **84**, I, 2026 (1913); *C. A.*, **7**, 2226 (1913).

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(5) (a) J. B. Levy, R. W. Taft, Jr., and L. P. Hammett, *ibid.*, **75**, 1253 (1953); (b) E. L. Purlee and R. W. Taft, Jr., *ibid.*, **78**, 5807 (1956).

(6) *Org. Syntheses*, **31**, 82 (1951).

(7) J. D. Roberts and C. W. Sauer, THIS JOURNAL, **71**, 3925 (1949).

(8) *Cf.* M. J. Murray and E. H. Stevenson, *ibid.*, **66**, 812 (1944).