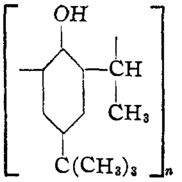
2. Koresin reacts with approximately one mole of bromine per structural unit of the resin. The bromine introduced is labile toward heat and toward alkali.
3. Acetylation of Koresin indicates approximately one free hydroxyl group per structural unit.
4. The probable structure of Koresin is



Chattanooga, Tenn. Received December 1, 1947

[Contribution from the Noyes Chemical Laboratory, University of Illinois]

The Mechanism of the Methane Fermentation

By A. M. Buswell and F. W. Sollo, Jr.[1]

Three possible mechanisms for the methane fermentation of acetic acid are suggested by the findings and reasoning of previous workers in this field. From the work of Omelianskii[2,3] and Söhngen[4,5] we would expect a preliminary decomposition of the acetic acid to hydrogen and carbon dioxide, with subsequent reduction of carbon dioxide to methane by the hydrogen. Barker's work[6] indicates that carbon dioxide would be directly reduced to methane and the acetic acid thereby oxidized to carbon dioxide. The reasoning of Buswell and Neave[7] leads to simple decarboxylation as the mechanism.

The first mechanism was based on the similarity of the hydrogen and methane fermentations, but the low concentration of hydrogen found in the gas from the methane fermentation of acetic acid is evidence against this mechanism. If we examine the data of Symons and Buswell[8] we find that only 3.5 liters of methane was formed over a period of one hundred days with regular circulation of hydrogen and carbon dioxide through a culture of 2.0 liters total volume. In the same time, such a culture fermenting acetic acid could be expected to form 75[8,9] liters of methane.

The second mechanism avoids this weakness by implying a direct reduction of the carbon dioxide, without the intermediate stage of free hydrogen. This mechanism seems rather indirect and involved, but parallels that found by Barker in the oxidation of alcohols.

The last mechanism appears to be the simplest and most direct. A similar reaction *in vitro* is the chemical decarboxylation of sodium acetate with sodium hydroxide. The internal oxidation reduction could be effected by the transfer of the hydrogen atom from the carboxyl to the methyl group. Evidence against this mechanism is found in Thayer's work.[10] He reasoned that this reaction was a decarboxylation, and that the fermentation of propionic and butyric acids should yield ethane and propane. However, his results were entirely negative, for no hydrocarbon other than methane was found. This has been confirmed in all work where the gas was analyzed. Therefore, if we are to accept this mechanism of simple decarboxylation for acetic acid, it must be as a special case, not applicable to the higher fatty acids.

Barker, Ruben and Kamen[11] found evidence for the reduction of carbon dioxide in the fermentation of acetic acid through the use of $C^{11}O_2$, but they stated that the radioactive methane found might have been due to the presence of methanol carried over with the inoculum. It should also be mentioned that these workers were using a pure culture of *Methanosarcina methanica*, and that even if methane should be formed by reduction of carbon dioxide in that case, that might not be the predominant mechanism in the general methane fermentation where a mixed culture is used.

This question of the mechanism of fermentation of acetic acid is of more than academic interest. Culture failure is almost invariably accompanied by, or preceded by, the accumulation of high concentrations of volatile organic acids, largely acetic. Thus any information concerning this mechanism might lead to methods of treatment or operation which would alleviate or prevent this accumulation of acids and possibly the failure of many cultures.

It may be seen at once that if the carbon of the carbon dioxide were marked isotopically, this question of mechanism could be settled. If either of the mechanisms involving reduction of carbon dioxide were involved, the methane produced should be similarly marked. If the reaction were a simple decarboxylation, the methane should not be so marked.

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(1) Present address: National Aluminate Corporation, 6216 W. 66th Place, Chicago, Illinois.

(2) W. Omelianskii, *Zentr. Bakt.*, II Abt., **8**, 193, 225, 257, 289, 321, 353, 385 (1902).

(3) W. Omelianskii, *ibid.*, II Abt., **11**, 369 (1904).

(4) N. L. Söhngen, *Rec. trav. chim.*, **29**, 238 (1910).

(5) N. L. Söhngen, *Proc. Roy. Acad. Amsterdam*, **8**, 327 (1905).

(6) H. A. Barker, *Arch. Mikrobiol.*, **7**, 404 (1936).

(7) A. M. Buswell and S. L. Neave, Ill. State Water Survey, Bull. No. 30, 1930.

(8) Ill. State Water Survey, Bull. No. 32, 1936, p. 47.

(9) D. Tarvin and A. M. Buswell, This Journal, **56**, 1751 (1934), p. 1752, Table I.

(10) L. A. Thayer, *Bull. Am. Assoc. Petroleum Geol.*, **15**, 441 (1931).

(11) H. A. Barker, S. Ruben and M. D. Kamen, *Proc. Natl. Acad. Sci.*, **26**, 426 (1940).

Radioactive C^{14} was chosen as the most easily handled of the carbon isotopes which could be used for this purpose.

Experimental

The radioactive carbon was obtained through the Manhattan district in the form of barium carbonate. This material was converted to sodium carbonate by acidification with perchloric acid and absorption of the carbon dioxide in a solution of sodium hydroxide. The solution resulting was found to yield 3.24 × 10^6 counts per minute per ml. One ml. of this solution was added to each culture before feeding and incubating.

The fermentations were carried out in 30-ml. round-bottomed flasks, connected to brine displacement gas collectors. Fifteen ml. of inoculum was taken from a digester which had been fermenting acetic acid for several months. To this was added 1.00 ml. of the radioactive sodium carbonate solution, and 1.0 ml. of 1.0% acetic acid. To the first three cultures, similar portions of acetic acid were added after two days of incubation, and again after the third day. The fourth culture was fed only once and analyzed after two days.

The first culture was analyzed after four days of incubation. The other two were kept in the incubator until they were analyzed, total incubation time being six days for the second and twelve days for the third.

The culture to be analyzed was acidified and boiled to free the dissolved carbon dioxide and to drive the gas over into the gas collector. Oxygen was added and after mixing, the sample was drawn through a train in which the carbon dioxide was first absorbed on carbon dioxide free Mikobite. A catalyst[12] of copper and cobalt oxides impregnated on porcelain, kept at 550°, effected the oxidation of the methane to carbon dioxide and water. This carbon dioxide was similarly absorbed. The carbonate was washed off the respective portions of Mikobite with hot water and precipitated with barium nitrate.

These operations were carried out in a carbon dioxide free atmosphere.

The precipitated barium carbonate was suspended in ethanol and deposited on an aluminum sample pan, by transferring to a brass tube, at the bottom of which was fastened the sample pan. The alcohol was evaporated under an electric heat lamp.

The samples thus prepared were counted with a conventional type Geiger–Mueller counter. The tube used was a pressure seal type mica window counter (Radiation Counter Laboratories, Mark 1, Model 2A, window thickness 2.19 mg. per sq. cm.). Background counts averaged from 40 to 45 counts per minute.

Correction for geometry was unnecessary, since only one geometry was used, and no correction for absorption by the window was made since the same tube was used throughout. The data of Yankwich, Rollefson and Norris[13] were used to calculate the correction of self absorption.

The specific activity of the methane was calculated by dividing the total counts by the weight of barium carbonate derived therefrom. The average specific activity of the carbon dioxide was calculated by dividing the average of the initial and final counts by the average of the initial and final equivalent weights of barium carbonate. From these, the activity ratio between the methane and the carbon dioxide, on the basis of the activity of the barium carbonate resulting, was calculated.

The activity ratio was found to vary with the time of incubation. It was for this reason that the additional sample with short incubation time was analyzed. The results are listed in Table I below.

TABLE I

Culture number	1	2	3	4
Days incubated	4	6	12	2
Acetic acid fed (g.)	0.0315	0.0315	0.0315	0.0105
CO_2 at start (mg. $BaCO_3$)	102.5	103.2	110.4	95.2
CO_2 at end (mg. $BaCO_3$)	197.4	215.5	226.4	136.8
Total counts per minute added	3.24 × 10^5	3.24 × 10^5	3.24 × 10^5	3.24 × 10^5
Activity of CO_2 at end (total counts per min.)	....	2.58 × 10^5	2.48 × 10^5	2.50 × 10^5
Average activity of CO_2 (counts/min./mg. $BaCO_3$)	1913	1805	1705	2474
Methane produced (mg. $BaCO_3$)	94.9	112.3	116.0	41.6
Total activity of CH_4 (counts/min.)	3415	6324	9903	549
Activity of CH_4 (counts/min./mg. $BaCO_3$)	36	56.3	85.4	13.2
Activity of CH_4/activity of CO_2	0.0188	0.0312	0.0501	0.0053

Discussion

If the mechanism of the fermentation of acetic acid involved the reduction of carbon dioxide to methane, the barium carbonate derived from the methane should have the same specific activity as the carbon dioxide had during the fermentation. The data show that the activity of the methane is only a few hundredths of that of the carbon dioxide, which makes it immediately apparent that the methane is derived from the acetic acid itself.

Calculation of the average activity of the carbon dioxide is an approximation, since we do not have an accurate measure of the activity at the various stages of methane production. The average figure used for this activity was thought to be the nearest estimate possible with the data obtained. It must be noted though, that even if we assumed the lowest possible activity, that found at the end of the incubation period, the ratio of the activities would be only slightly raised. For example, in culture no. 2, where the ratio found was 0.0312, use of the lower total activity would raise this figure to only 0.0358.

In Fig. 1, the ratio of activities is plotted against time of incubation. This curve indicates that there is a lag period, with a subsequent gradual rise in the activity ratio. It seems likely that from this we can assume that very little carbon dioxide is directly reduced to methane in this fermentation, and that the source of the radioactive methane is an indirect fixation of carbon dioxide, with subsequent decomposition of the material formed therefrom. Whether we assume fixation into cell

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(12) I. F. Walker and B. E. Christensen, *Ind. Eng. Chem., Anal. Ed.*, **7**, 9 (1935).

(13) P. E. Yankwich, G. K. Rollefson and T. H. Norris, *J. Chem. Phys.*, **14**, 131 (1946).

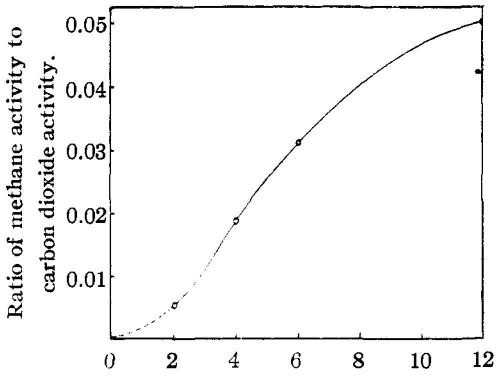


Fig. 1.—Relation of activity ratio to time of incubation.

material with later autolysis, or the formation of endogenous compounds, makes little difference. In any case we must conclude that the predominant reaction in the methane fermentation of acetic acid does not involve reduction of carbon dioxide.

This work indicates that the methane is produced by some mechanism other than the reduction of carbon dioxide, and must therefore be derived from the acetic acid. A simple decarboxylation seems to be the most likely mechanism, but is not definitely established. It is still possible that some preliminary condensation might take place, with subsequent decomposition of the condensation product, but this point cannot be proved by this method of attack.

Summary

It was suggested by previous workers that the methane fermentation of acetic acid might proceed by reduction of carbon dioxide to methane, as was shown to be the case for several alcohols.

In order to determine whether or not this was the case, radioactive C^{14} was used to mark the carbon atom of the carbon dioxide. The gases resulting from the fermentation were separated and converted to barium carbonate for measurement of the radioactivity.

By comparing the activity of the methane and the carbon dioxide, it was shown that only a very small portion of the methane was derived from the carbon dioxide. By studying the relation of the amount of methane formed by reduction of carbon dioxide to the time of incubation, a correlation was found which was interpreted as indicating a slow reduction unassociated with the general fermentation, such as the formation of cell substance and subsequent autolysis. From this it was concluded that the acetic acid was fermented entirely, or very nearly so, without reduction of carbon dioxide, and that the methane is predominantly derived from the acetic acid and not from carbon dioxide.

URBANA, ILLINOIS RECEIVED OCTOBER 27, 1947

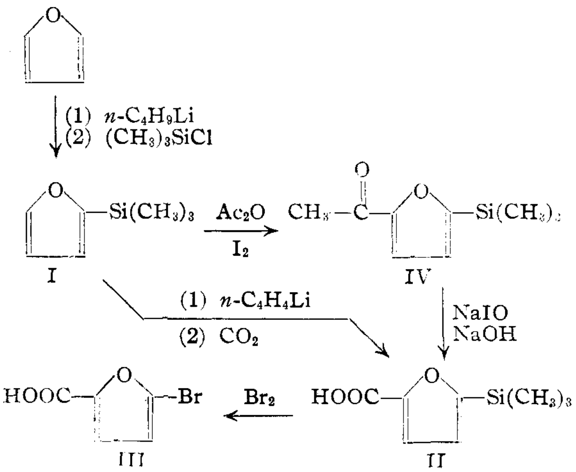
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF PURDUE UNIVERSITY]

Acylation Reactions with Organosilicon Compounds[1]

BY ROBERT A. BENKESER AND ROBERT B. CURRIE[2]

Apparently, the only attempt to carry out a Friedel–Crafts reaction involving an organosilicon compound is the work of Kipping.[3] He reported that if a compound like tetraphenylsilane is heated with aluminum chloride the only product isolated is silicon tetrachloride (80% yield). Viewed from the generalized Lewis concept of acids[4] this is not surprising since it has long been known that the aromatic carbon–silicon bond is readily cleaved by acidic reagents.[5–7] The electropositive silicon atom tends to combine with a more electronegative element than carbon when given the opportunity.

It has now been found that using the mild catalyst iodine, an acylation reaction can be carried out with certain organosilicon compounds. In the experiments herein reported, 2-thienyltrimethylsilane and 2-furyltrimethylsilane were acetylated with acetic anhydride at 50°. The yields were of the order of 20–25%. The equations indicate the general sequence of reactions with furan



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[1] A portion of this work is abstracted from the thesis submitted by Robert B. Currie to Purdue University in partial fulfilment of the requirements for the degree of Master of Science, August, 1947.

[2] Present address: Merck and Company, Rahway, New Jersey.

[3] Evison and Kipping, *J. Chem. Soc.*, 2774 (1931).

[4] Lewis, *J. Franklin Inst.*, **226**, 293 (1938).

[5] Ladenburg, *Ann.*, **173**, 143 (1874).

[6] Kipping and Lloyd, *J. Chem. Soc.*, **79**, 449 (1901).

[7] Kipping, *ibid.*, **91**, 223 (1907)