The ether solution of acid-soluble material gave 67 g. of liquid boiling at 100–110° (35 mm.). Fractionation resulted in 60 g. (65% yield) of allylmethylisoamoxymethylcarbinamine; b. p. 89–90° (8 mm.); d^{20}_4 0.8383; $n^{20}D$ 1.4355; *MR* calcd. 57.60; *MR* found 57.74.

Anal. Calcd. for $C_{11}H_{23}NO$: N, 7.56. Found: N, 7.60.

A picrate was obtained in 92% yield and melted at 104° (cor.) after recrystallization from benzene–petroleum ether mixture.

Anal. Calcd. for $C_{17}H_{26}N_4O_8$: N, 13.52. Found: N, 13.61.

From the ether solution of acid-insoluble material was obtained 14.3 g. of a liquid boiling at 60–106° (35 mm.). No sharply boiling fractions could be observed on redistillation; however, a 2-g. fraction, b. p. 65–85° (26 mm.), gave on treatment with 3,5-dinitrobenzoyl chloride a small yield of 3,5-dinitrobenzoyl ester which melted at 61° (cor.). This ester proved to be isoamyl 3,5-dinitrobenzoate.

Anal. Calcd. for $C_{12}H_{14}N_2O_6$: N, 9.93. Found: N, 9.80.

In addition to the above, there was collected a 6.5-g. fraction which boiled at 90–104° (26 mm.) and formed a solid semicarbazone in good yield. This derivative was quite soluble in water but crystallized well from petroleum ether; m. p. 60° (cor.). Presumably, it is the semicarbazone of methyl isoamoxymethyl ketone.

Anal. Calcd. for $C_9H_{19}N_3O_2$: N, 20.88. Found: N, 21.05.

Methylisoamoxymethyl-*n*-propylcarbinamine.—In preparation of this amine, 25.6 g. of allylmethylisoamoxymethylcarbamine in 100 cc. of ethyl alcohol was reduced in the presence of 0.1 g. of Adams catalyst and hydrogen under 70 cm. pressure. The yield was 23.1 g. (90%); b. p. 97° (8 mm.); d^{20}_4 0.8264; $n^{20}D$ 1.4280; *MR* calcd. 58.06; *MR* found 58.30.

Anal. Calcd. for $C_{11}H_{25}NO$: C, 70.53; H, 13.45; N, 7.48. Found: C, 70.39; H, 13.50; N, 7.57.

The picrate was recrystallized from benzene–petroleum ether (93% yield) and then melted at 86° (cor.).

Anal. Calcd. for $C_{17}H_{28}N_4O_8$: N, 13.46. Found: N, 13.60.

Summary

1. The two isomeric ethoxypropionitriles have been shown capable of reaction with two equivalents of allylmagnesium bromide to form the corresponding diallylethoxyethylcarbinamines. The latter are reduced readily by catalytic hydrogenation to the di-*n*-propylethoxyethylcarbinamines.

2. Although 1-ethoxypropionitrile and *n*-propylmagnesium bromide interact to yield an adduct with which allylmagnesium bromide reacts to yield allyl-1-ethoxyethyl-*n*-propylcarbinamine, the isomeric 2-ethoxypropionitrile does not evidence a similar behavior. The lesser activity of 2-ethoxypropionitrile is shown further by its failure to react smoothly with *n*-propylmagnesium bromide to yield an anticipated ketone.

3. The adducts formed from two additional alkoxyacetonitriles, by interaction with methylmagnesium halides, also react with allylmagnesium bromide to yield carbinamines.

4. These carbinamines lower blood pressure in a pitted cat but not to a useful degree.

AUSTIN, TEXAS RECEIVED FEBRUARY 23, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

The Production of Acetylmethylcarbinol by the Action of *Acetobacter suboxydans* upon 2,3-Butylene Glycol[1]

BY E. I. FULMER, L. A. UNDERKOFLER AND A. C. BANTZ

Previous communications from these laboratories have presented data on the production of keto-compounds by the action of *Acetobacter suboxydans* upon sorbitol,[2] glycerol,[3] erythritol,[4] mannitol[5] and *i*-inositol.[6] The present paper deals with the production of acetylmethylcarbinol by the action of this organism upon 2,3-butylene glycol.

Kling[7] obtained a 50% yield of acetylmethylcarbinol after a thirty-three day fermentation of 2,3-butylene glycol by *Mycoderma aceti*. Visser't Hooft[8] reported a 77% yield of the carbinol from the glycol by the action of *Acetobacter suboxydans*. Both workers used the butylene glycol obtained by the fermentative action of *Aerobacter aerogenes* and assumed that they were dealing only with

(1) This work was supported by a grant from the Industrial Science Research funds of the Iowa State College for studies on the fermentative utilization of agricultural products.

(2) E. I. Fulmer, J. W. Dunning, J. F. Guymon and L. A. Underkofler, THIS JOURNAL, **58**, 1012 (1936).

(3) L. A. Underkofler and E. I. Fulmer, *ibid.*, **59**, 301 (1937).

(4) R. L. Whistler and L. A. Underkofler, *ibid.*, **60**, 2507 (1938).

(5) E. I. Fulmer, J. W. Dunning and L. A. Underkofler, *Iowa State Coll. J. Sci.*, **13**, 279 (1939).

(6) J. W. Dunning, E. I. Fulmer and L. A. Underkofler, *ibid.*, **15**, 39 (1940).

(7) A. Kling, *Ann. chim.*, [8] **5**, 471 (1905).

(8) F. visser't Hooft, "Biochemische onderzoekingen over het Geslacht Acetobacter," Thesis, Delft, 1925.

the *d*- and *l*- forms of 2,3-butylene glycol. Based upon his 50% conversion, Kling concluded that only the *l*-glycol was attacked. On the basis of his 77% conversion, visser't Hooft concluded that both forms of the glycol were oxidized.

The 2,3-butylene glycol produced by the fermentative action of *Aerobacter aerogenes* is now known to contain a mixture of the stereoisomers, with the *meso*-glycol predominating. The glycol employed in the present investigation showed a slight dextrorotatory power and from freezing point data[9] was found to contain about 90% of the *meso*-glycol.

Experimental

Methods.—The culture of *Acetobacter suboxydans* was originally obtained from the American Type Culture Collection listed as No. 621. The stock cultures were carried on yeast extract-glycerol-agar slants. The stock culture was transferred to a 5% glycerol-0.5% yeast extract (Difco powdered product) medium and kept active by transferring to a fresh medium each forty-eight hours. All cultures used for inoculations had grown for twenty-four hours on a 5% 2,3-butylene glycol-yeast extract medium. The media were adjusted to *p*H 6.0 ± 0.1, which is optimum for the growth of the organism,[10] and sterilized for fifteen minutes at 15 lb. steam pressure. The incubation temperature was 28°. Unless otherwise specified, the fermentations were carried out using 10 ml. of medium in each 50-ml. Erlenmeyer flask. In each case the inoculum consisted of 6 drops of culture per 10 ml. of medium. All fermentations were run in duplicate and the values reported are the average for the duplicate determinations.

The course of the conversion of the glycol to acetylmethylcarbinol was followed by determining the content of reducing substance developed in the media, using a modified Shaffer-Somogyi method developed in these laboratories.[11]

The amount of diacetyl produced in the fermentations was found to be negligible. Corrections were made in all cases for reducing materials present in the media at the beginning of the fermentations.

The Development of the Medium.—It was found that the culture of *A. suboxydans* could not be carried beyond the fourth or fifth transfer in the medium containing 2,3-butylene glycol. The same situation had been met with in previous investigations in using *i*-inositol as the substrate.[6] However, in the previous studies it was found that the addition of very low concentrations of sorbitol, glycerol, erythritol, dextrose or mannitol to the inositol medium permitted indefinite subculture of the organism with almost complete oxidation of the inositol.

Data are presented in Table I showing the effect of the addition of 0.25% of sucrose, sorbitol, dextrose or maltose upon the yield of acetylmethylcarbinol from a 5% butylene glycol-yeast extract medium. It is evident that the maltose was the most effective of the substrates tested; the maltose permitted the indefinite subculture of the organism and oxidation of the glycol. In Table II are given data on the effect of several concentrations of maltose upon the yield of the carbinol from a 10% glycol medium. Good yields of the carbinol were obtained in a medium containing 10% glycol; very low yields were obtained, in similar experiments, in media containing 20% glycol.

TABLE I

THE EFFECT OF SEVERAL SUBSTRATES (0.25 G./100 ML. OF MEDIUM) UPON THE YIELD OF ACETYLMETHYLCARBINOL FROM A 5% 2,3-BUTYLENE GLYCOL MEDIUM

	% conversion of the glycol to the carbinol, in hours			
Substrate	24	48	72	96
Sucrose	13	22	25	20
Sorbitol	13	18	20	24
Dextrose	36	61	75	75
Maltose	27	72	92	95

TABLE II

THE EFFECT OF VARYING CONCENTRATIONS OF MALTOSE UPON THE YIELD OF ACETYLMETHYLCARBINOL FROM A 10% 2,3-BUTYLENE GLYCOL MEDIUM

	% conversion of the glycol to the carbinol in hours			
Maltose, g. per 100 ml.	24	48	72	96
0.00	6	8	9	12
.12	8	16	38	47
.25	..	23	60	66
.50	7	32	75	89
1.00	..	58	..	90

In a typical larger scale fermentation 2.5 liters of a medium, containing 10% of 2,3-butylene glycol, 0.5% yeast extract and 0.5% maltose, were used in a 4-liter Erlenmeyer flask. The *p*H of the medium was adjusted to a value of 6.0 and it was sterilized in the usual manner. The medium was inoculated with 100 ml. of a twenty-four-hour culture of *Acetobacter suboxydans* grown on a butylene glycol-yeast extract-maltose medium, and the inoculated medium was aerated vigorously, using a single porous alundum ball, during the course of the fermentation. The yields of acetylmethylcarbinol after 24, 48, 76, 96, 120, 144 and 168 hours were, respectively, 18, 38, 73, 83, 90, 93 and 94%. It is evident that satisfactory yields of the carbinol were obtained but that the rate of conversion was slower than with the smaller volumes; more efficient aeration would no doubt materially increase the rate of the reaction.

The Recovery of the Acetylmethylcarbinol.—Fractional distillation gave unsatisfactory results due to the continuous distillation of water with the acetylmethylcarbinol even when an efficient fractionating column was employed. Extraction of the untreated fermented liquid with ether was very slow. A satisfactory method was finally developed, however, which is as follows: The fermented medium is clarified with diatomaceous earth and filtered. The clear filtrate is saturated with sodium sulfate and extracted with ether in a continuous extraction apparatus. With the above procedure over 91% of the carbinol and 99% of the unfermented glycol are extracted in thirty hours. The ether is removed from the extract by distilla-

(9) C. E. Wilson and H. J. Lucas, THIS JOURNAL, **58**, 2396 (1936).

(10) L. A. Underkofler, A. C. Bantz and W. H. Peterson, *J. Bact.* **45**, 183 (1943).

(11) L. A. Underkofler, J. F. Guymon, M. M. Rayman and E. I. Fulmer, *Iowa State Coll. J. Sci.*, **17**, 251 (1943).

tion and the residue, containing the acetylmethylcarbinol and the unfermented glycol, is carefully fractionated. The fraction boiling between 135 and 150° is further purified by the use of an efficient fractionating column. The final fraction of the acetylmethylcarbinol obtained in a typical experiment had a boiling range of 142–144°, and the refractive index at 15° was 1.4192 as compared with the literature value of 1.4194.

The Properties of the Unfermented Glycol.—The unfermented glycol fraction recovered by the ether extraction and subsequent fractionation was purified by three distillations and was entirely free from the carbinol. The boiling range of the glycol was 179–182°. The angle of rotation of the glycol using the D line of sodium was $[\alpha]^{25}D$ +10.15. The highest rotations previously reported for *d*-2,3-butylene glycol are $[\alpha]^{25}D$ +5.0 by Böeseken and Cohen[12] and $[\alpha]^{25}D$ +6.9 by Chappell.[13] For the 2,3-butylene glycol used in the fermentations of the present investigation, produced by the action of *Aerobacter aerogenes* upon sugar, it was $[\alpha]^{25}D$ +1.0°. The freezing point of this glycol was 27°, which, according to the data of Wilson and Lucas,[9] corresponds to a mixture containing about 90% of the *meso*-glycol. The results of the above experiments indicate that the *Acetobacter suboxydans* preferentially attacks the *meso*-glycol and that the original glycol contained little or none of the *l*-form. Experiments are being conducted for the production of larger amounts of the *d*-glycol and its further purification.

Preliminary data indicate the feasibility of a dual fermentation for the production of acetylmethylcarbinol. The carbohydrate medium is first fermented with *Aerobacter aerogenes* to produce the 2,3-butylene glycol. The fermented medium is then sterilized, any needed adjustments in *p*H are made, and the medium inoculated with *Acetobacter suboxydans* to produce the acetylmethylcarbinol. By this procedure it is not necessary to isolate the glycol before subjecting it to the carbinol fermentation.

(12) J. Böeseken and R. Cohen, *Rec. trav. chim.*, **47**, 839 (1928).

(13) C. H. Chappell, "A Study of 2,3-butylene glycol and its derivatives," Thesis, Iowa State College, 1935.

Summary

1. A procedure has been described for the production of acetylmethylcarbinol by the action of *Acetobacter suboxydans* upon 2,3-butylene glycol. The yields of the carbinol are 90–94% of theory.

2. The unfermented 2,3-butylene glycol is dextrorotatory with an angle of rotation much higher than that previously reported in the literature. These results indicate that the organism attacks the *meso*-glycol preferentially and that the glycol produced by the action of *Aerobacter aerogenes* consists of the *meso*-glycol and *d*-glycol with little or none of the *l*-glycol.

AMES, IOWA RECEIVED APRIL 9, 1943

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, NO. 931]

The Equilibrium of Gaseous Dibromoethylenes

BY RICHARD M. NOYES AND ROSCOE G. DICKINSON

The kinetics of the iodine catalyzed liquid phase isomerization of the *cis* and *trans* 1,2-dichloroethylenes have been studied by Wood and Dickinson,[1] and the vapor phase equilibrium has been investigated by Wood and Stevenson.[2] However, much less work has been done on the dibromoethylenes. Olson and Maroney[3] have studied the liquid phase equilibrium at 25 and at 150°, but no vapor phase work has been done. Since the techniques had already been developed in this Laboratory in connection with the work on the dichloroethylenes,[2] it was felt that it might be instructive to extend the study to the dibromoethylenes.

Experimental

Materials.—The iodine used as a catalyst in the reaction was sublimed from potassium iodide–iodine mixture and resublimed.

The dibromoethylene was prepared by adding commercial tetrabromoethane to a mixture of zinc and alcohol as directed by Van de Walle.[4] The isomers were separated by fractional distillation with absolute alcohol, the fractionation being followed by the index of refraction of the distillate. The dibromoethylene was removed from the alcohol by extracting with six molar sulfuric acid, washed with water, and dried with potassium carbonate.

The dielectric constant of *cis*-dibromoethylene obtained by the above method was not appreciably affected by subsequent attempts at purification. The dielectric constant of the *trans* isomer could be lowered somewhat by crystallization from methanol at dry-ice temperatures. The dielectric constants at 25° and 1.7 megacycles were 2.47 and 6.96 for the *trans* and *cis* isomers, respectively.

The *cis* isomer was quite stable, but the *trans* turned yellow on standing in air. The dielectric constant of *trans*-dibromoethylene exposed to the air changed rapidly and reached a value corresponding to 62% *cis* in agreement with the equilibrium observation of Olson and Maroney.[3] It was found that both isomers could be stored indefinitely under nitrogen without appreciable isomerization.

Procedure.—The composition of isomeric mixtures was determined by means of the dielectric constant. Mix-

(1) Reuben E. Wood and Roscoe G. Dickinson, THIS JOURNAL, **61**, 3259–3263 (1939).

(2) Reuben E. Wood and D. P. Stevenson, *ibid.*, **63**, 1650–1653 (1941).

(3) A. R. Olson and William Maroney, *ibid.*, **56**, 1322 (1934).

(4) H. Van de Walle, *Bull. soc. chim. belg.*, **27**, 209–217 (1913).