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DEGRADATION OF D-GLUCOSE WITH ACETIC ACID AND METHYLAMINE*

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ABSTRACT

Thirteen products have been isolated from degradation of D-glucose with methylamine and acetic acid. Two compounds are responsible for the caramel odor observed in this degradation and have been identified as acetylformoin and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone. Two new compounds, 2-(2-hydroxyacetyl)-1-methylpyrrole and 5-hydroxymethyl-1-methylpyrrole-2-carboxaldehyde are reported for the first time. Eleven of the 13 compounds isolated are derivatives of furan or pyrrole.

INTRODUCTION

Degradation products from carbohydrates in foods and model systems have been studied for many years[1]. Carbohydrates are known to produce color and off-flavors in many fruits and foodstuffs through nonenzymic browning[2]. Tatum and co-workers[3] identified 16 degradation products in dehydrated orange-juice powder (approximately 95% carbohydrates). Shaw and co-workers isolated and identified 13 compounds from the acid-catalyzed degradation of D-fructose[4] and 18 compounds from the base-catalyzed degradation of D-fructose[5]. Severin and Seilmeier[6] reported a new compound formed in the degradation of D-glucose with acetic acid and methylamine. This compound had the empirical formula $C_6H_6O_4$ and was identical with a compound isolated by Shaw, Tatum, and Berry[4]. Both groups, however, assigned incorrect structures to this compound. The correct structure was shown by Mills, Weisleder, and Hodge[7] to be 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (**1**). The present paper reports the isolation and identification of other degradation products, extractable by ethyl acetate, formed from D-glucose in the presence of acetic acid and methylamine.

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RESULTS AND DISCUSSION

Thirteen compounds were isolated after degradation of D-glucose with methylamine and acetic acid (Table I). Two of these componds, acetylformoin (**2**) and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (**8**), have intense, caramel-like odors[4,5]. We have shown that acetylformoin as an impurity (see experimental section) was responsible for the caramel-like odor of the 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (**1**) isolated by Severin and Seilmeier[6]. When degradation by a procedure *A* was made in triplicate and the extracts combined, compound **1** was obtained in 0.1% yield. If a degradation procedure *B* were used, compound **1** could be isolated in 0.25% yield (see experimental section). Table I lists the yield of each product from degradation procedure *B*, as determined by g.l.c. peak areas.

TABLE I

COMPOUNDS ISOLATED FROM DEGRADATION OF D-GLUCOSE WITH ACETIC ACID AND METHYLAMINE

Compound		R_T[a] (min)	R_F *by t.l.c. (15 cm)*	*Color with anisaldehyde spray*	*Yield*[b] (%)
1	2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	65	0.33	Yellow	0.36
2	4-Hydroxy-2,3,5-hexanetrione (acetylformoin)	15.5	0.72	White to red	[c]
3	2-(2-Hydroxyacetyl)-1-methylpyrrole	58	0.66	Red brown	0.04
4	3,5-Dihydroxy-2-methyl-4*H*-pyran-4-one	67.5	0.40	White	0.02
5	5-Hydroxymethyl-1-methylpyrrole-2-carboxaldehyde	88	0.40	Dark brown	0.30
6	2-Acetyl-1-methylpyrrole	23	0.98	Yellow red	[c]
7	5-Methyl-2-furfuryl acetate	19.8	0.94	Dark brown	[c]
8	2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone	49.6	0.62	Brown	[c]
9	Propionic acid	16.4			[c]
10	1-Methylpyrrole-2-carboxaldehyde	21	0.96	Yellow brown	[c]
11	5-Hydroxymethyl-2-furaldehyde	80	0.38	Green	0.04
12	Furfuryl alcohol	22	0.50	Purple	[c]
13	5-Methyl-2-furfuryl alcohol	26.3	0.66	Brown	[c]

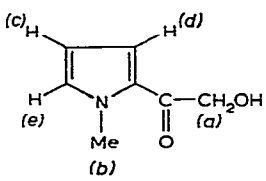
[a]Column 1. [b]Determined from g.l.c. peak areas (width at half-height × height). [c]Less than 0.01%.

The identity of propionic acid (**9**), 1-methylpyrrole-2-carboxaldehyde (**10**), furfuryl alcohol (**12**), and 5-hydroxymethyl-2-furaldehyde (**11**) was confirmed by comparing their i.r. spectra, mass-spectral fragmentation patterns and g.l.c. retention times with those of authentic samples.

5-Methyl-2-furfuryl acetate (**7**), 2-acetyl-1-methylpyrrole (**6**), and 5-methyl-2-furfuryl alcohol (**13**) were identified similarly, by i.r. and mass spectroscopy and by t.l.c.

2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (**8**) and acetylformoin (**2**) were likewise identified with reference samples[4,5] by i.r. and mass spectroscopy, and by g.l.c. retention times (R_T).

Two new compounds (**3** and **5**) are reported here for the first time.



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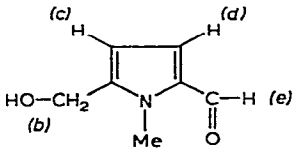

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2-(2-Hydroxyacetyl)-1-methylpyrrole (**3**) was the structure assigned to **3** from spectral data alone. An attempt to synthesize compound **3** from 2-(2-hydroxyacetyl)-furan and methylamine failed. 5-Hydroxymethyl-1-methylpyrrole-2-carboxaldehyde (**5**) was synthesized from 5-hydroxymethyl-2-furaldehyde and methylamine to confirm its identity (see experimental section). Kato[8] isolated 1-substituted pyrrole-2-carboxaldehydes from similar degradations, with D-xylose and L-rhamnose, but was unsuccessful in obtaining the corresponding pyrrole derivatives from D-glucose. In the present study, four pyrroles (**3**, **5**, **6**, and **10**) were identified.

3,5-Dihydroxy-2-methyl-4*H*-pyran-4-one (**4**) was identified by comparing its i.r. and u.v. spectra with data published by Terada and co-workers[9]. Shaw and co-workers[10] oxidized 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (**1**) with chromium trioxide–pyridine and obtained compound **4**, identical by i.r. and mass spectra and by g.l.c. retention time with compound **4** isolated in this study. Terada reported m.p. 184–4.5° for this compound, whereas we found m.p. 156–156.5°, This compound apparently exhibits polymorphism. In order to obtain more evidence for compound **4** it was converted into its diacetate (**14**). An attempt to reduce the carbonyl group of the diacetate with sodium borohydride failed, and a monoacetate (**15**) was obtained. The carbonyl group of the dialcohol (**4**) absorbed at 1540 cm^{-1}. The monoacetate absorbed at 1610 cm^{-1} and the diacetate at 1670 cm^{-1}. This is a total shift of 130 cm^{-1} from the dialcohol to the diacetate. The dialcohol is not soluble in carbon tetrachloride, chloroform, dichloromethane, or carbon disulfide, and it was therefore not possible to observe a shift in the i.r. spectrum to prove whether or not intramolecular hydrogen-bonding occurs.

All of the compounds listed in Table I except propionic acid and **4** are compounds of the furan type or 1-methyl analogs of furans, and a mechanism for their formation has been postulated[11–14].

EXPERIMENTAL

General. — Melting points are uncorrected. U.v. spectra were recorded in abs. ethanol with a Cary Model 14 spectrophotometer. I.r. spectra were obtained with a Perkin–Elmer Model 137 Infracord spectrophotometer. Absorption peaks are designated in the following manner: s, strong; w, weak; m, moderate; and sh, shoulder. Low-resolution mass spectra were determined with a Bendix Model 3012 Time-of-Flight mass spectrometer. Mass spectral data are given as *m/e* values, and peak intensities are expressed, in parentheses, as percent of the most intense peak. High resolution mass spectra were determined with an A.E.I. Picker, ultrahigh-resolution mass spectrometer at Florida State University.

All n.m.r. spectra were determined with a Varian A-60 spectrophotometer. Chemical-shift values are given in δ p.p.m. and tetramethylsilane was used as an internal standard. G.l.c. analyses were performed with an F & M Model 810 instrument equipped with a 10 to 1 effluent splitter, and samples were collected in capillary tubes cooled with liquid nitrogen. The temperature at the injection port was 210° and at the exit port 200°. The columns and programs were as follows: Column *A*. A 9 ft × 0.25 in, stainless-steel column packed with Carbowax 20M (20% on 60–80 mesh Gas-Chrom P) was operated at a helium flowrate of 180 ml/min. The temperature program was as follows: initial temperature 80°, for 6 min, and then the temperature was increased to 130° at 6 min, 135° at 14 min, 140° at 24 min, 155° at 30 min, 180° at 46 min, 190° at 56 min, 200° at 64 min, 220° at 74 min, 240° at 82 min. All temperature increases were at the rate of 60° per min, and all times were taken from the moment the sample was injected on the column. Column *B*. A 9 ft × 0.25-in stainless-steel column packed with SE-30 (25% on 60–80 mesh Gas-Chrom P) was operated at a helium flowrate of 180 ml/min. The temperature program was as follows: 80° at start, after four min begin program 4° per min to 220°.

T.l.c. was effected with 2 × 8-in glass plates coated with 10–30 μm mesh Bio Sil A. Plates were developed in 200:47:15:1 benzene–ethanol–water–acetic acid[15] (upper phase). Compounds were detected with an ethanol–anisaldehyde–sulfuric acid mixture 36:2:2 by volume. The plates were heated for 4 min at 140° for development of color.

Degradation of D-*glucose*[6]. — *Degradation procedure* A. A mixture of aqueous methylamine (40%, 20 ml, 0.28 mole) acetic acid (14 ml), and D-glucose (130 g, 0.72 mole) in 700 ml of water was refluxed for 2 h on a steam bath. The cooled mixture was extracted 3 times with 300-ml portions of ethyl acetate, and the dried (sodium sulfate) extract was concentrated at 60° to remove the ethyl acetate. Extracts obtained by this procedure were either distilled or examined by g.l.c. Severin and Seilmeier[6] stated that an oil containing 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one distilled at 80° and 0.1 torr. We found that it distilled at 180° at 0.1 torr into a trap cooled with liquid nitrogen. If the side arm of the distillation apparatus were insulated with asbestos tape, the oil distilled at 155° and 0.1 torr. From this oil there separated a white solid after 30 min at room temperature. Recrystallization of the

solid from ethyl ether–cyclohexane gave 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one in 0.1% yield; m.p. 69–71°. Severin and Seilmeier reported m.p. 72–74°.

Degradation procedure B. A mixture of 40% aqueous methylamine (60 ml, 0.83 mole), acetic acid (42 ml), D-glucose (390 g, 2.2 moles), and 900 ml of water was refluxed for 2 h on a steam bath, and then the mixture was cooled, transferred to a 2-l beaker and 300 g of sodium chloride was added. The solution was extracted 4 times with 400-ml portions of ethyl acetate. The extract was dried (sodium sulfate) and concentrated at 70° to remove the ethyl acetate. A quantitative determination was made with this extract by g.l.c. with 5-hydroxymethyl-2-furaldehyde as a standard. The percent-yield data were calculated as moles product/moles D-glucose × 100 and are listed in Table I. These values represent proportions of each compound in the extract, as calculated from the g.l.c. curve, rather than amounts isolated pure.

Gas-liquid chromatography. — Separation of the extracts from degradation *A* or *B* on Column *A* yielded the compounds listed in Table I. A 25-mg sample of compound **1** (m.p. 69–71°) was subjected to further purification on Colunm *B*. Two compounds were isolated, acetylformoin[4] (**2**), which had an intense caramel-like odor and compound **1**, which no longer possessed a caramel-like odor but had a charred odor. Acetylformoin was present in trace quantities only, but enough material was trapped for comparison with an authentic sample[4] by t.l.c. and by i.r. and mass spectrometry.

When the total extract from degradation *A* or *B* was separated on Column *A*, acetylformoin (**2**) had a retention time of 15.5 min. There was also bleeding of this material from 48.5 min until 65 min into the run. When pure acetylformoin was separated under the same conditions, it had a retention time of 15.5 min and there was no bleeding at 48.5 to 65 min into the run. This result indicates that an intermediate was undergoing breakdown during the g.l.c. separation to give acetylformoin (**2**) at the higher temperatures and retention times.

2-(2-Hydroxyacetyl)-1-methylpyrrole (**3**). — The product, m.p. 49–50,5°, was assigned the structure **3** from spectral data; λ_{max}^{film} 3430s (OH), 3100sh, 2925w, 1740m, 1660s (C=O), 1534s, 1405s, 1385s, 1330s, 1270w, 1240m, 1222w, 1138m, 1110w, 1090m, 1063s, 1038s, 955s, 785m, 790w, 750s, 690m cm^{-1}; *m/e* 139 (70) (M^+), 108 (100) (M^+–CH_2OH), 80 (22) [M^+–(C=O)CH_2OH], 60 (22), 57 (57), 45 (39), 44 (85), 39 (52); high-resolution *m/e* 139.0610 (calc. for $C_7H_9NO_2$, 139.0632); n.m.r. data in $CDCl_3$: δ 4.60 (singlet, CH_2-*a*), 3.95 (CH_3-*b*), 6.14 (1 proton, 2 doublets, H-*e*), 6.87 (singlet, H-*d*), 6.92 (1-proton doublet, H-*e*).

The n.m.r. spectrum of compound **3** resemble those of compounds **5** and **6**. The i.r. and mass-spectral data for **3** are very similar to those for 2-(2-hydroxyacetyl)-furan[4]. An attempt to synthesize compound **3** from 2-(2-hydroxyacetyl)furan and methylamine failed.

*3,5-Dihydroxy-2-methyl-4*H*-pyran-4-one* (**4**). — M.p. 156–156.5°; n.m.r. data for **4** in $HCON(CD_3)_2$: δ 2.3 (Me), 8.01 (1-proton singlet), no OH signals observed; λ_{max} 289 (ε 7,930), 219 nm (8,613); λ_{max}^{KBr} 3100s (broad), 1648w, 1598s, 1540s (broad), 1440w, 1300s, 1225s, 1195s, 1118s, 1038w, 1017s, 942s, 882s, 785sh, 775s cm^{-1}; *m/e*

142 (100), 113 (9.5), 96 (8), 87 (7), 85 (15), 71 (17), 69 (9), 68 (32), 67 (12), 57 (12), 55 (21), 53 (11), 43 (44), 42 (13), 41 (14), 40 (21), 39 (19).

*3,5-Diacetoxy-2-methyl-4*H*-pyran-4-one diacetate* (**14**). — To 50 mg of compound **4** was added 250 μl of acetic anhydride, 2.5 ml of chloroform, and 0.1 g of fused sodium acetate, and the mixture was kept for 12 h at room temperature. Water (2 ml) was added and the mixture kept for 1 h at room temperature. The organic layer was separated and the water fraction was extracted again with 3 ml of chloroform. The combined organic layers were washed with 2 ml of M sodium hydroxide and 2 ml of water, dried, and the solvent removed, to give **14**, m.p. 102-102.5°; λ_{max} 257 (ε 10,923), 212 nm (10,546); λ_{max}^{KBr} 3100w, 1775s, 1670s, 1620m, 1430s, 1370s, 1350sh, 1270s, 1190s, 1042sh, 1022s, 980m, 945s, 912s, 888s, 860m, 790m, 778s, 732s cm^{-1}; n.m.r. data in $HCON(CD_3)_2$: δ 1.95 (singlet, 2 Me groups), 2.00 (singlet, Me) 8.15 (1-proton singlet); *m/e* 226 (3), 184 (13), 142 (37), 104 (6), 71 (6), 69 (6), 57 (12), 55 (14), 43 (100).

*3,5-Dihydroxy-2-methyl-4*H*-pyran-4-one (3 or 5)-monoacetate*[16] (**15**). — To 25 mg of **14** was added 25 mg of sodium borohydride in 10 ml of 90% methanol, and the mixture was kept for 3 h at 0°. Acetic acid (2 ml) and 10 ml of water were then added. The solvent was removed under vaccuum and the remaining material was extracted 4 times with 30-ml portions of chloroform. The dried (sodium sulfate) extract was evaporated and the solid remaining was recrystallized from ether to give white prisms; m.p. 132–135°, λ_{max}^{KBr} 3170s, 1770s, 1610s, 1430w, 1385s, 1365s, 1310s, 1265w, 1200s, 1170s, 1118s, 1030w, 1025s, 945s, 885m, 855s, 788m, 767s, 730w, 695w cm^{-1}; *m/e* 184 (6), 142 (23), 97 (6), 83 (7), 71 (11), 69 (13), 57 (26), 55 (28), 43 (100), 41 (42).

5-Hydroxymethyl-1-methylpyrrole-2-carboxaldehyde[11] (**5**). — A mixture of 5-hydroxymethyl-2-furaldehyde (2.04 g, 6.2 mmoles) methanol (25 ml) and 40% aqueous methylamine (25 ml, 0.32 mole) was heated in a stainless-steel bomb for 24 h at 140°. The resultant solution was concentrated. Water (10 ml) was added and the pH was adjusted to 4.0 with hydrochloric acid, and then the solution was extracted three times with 25 ml of ethyl acetate and two times with 25 ml of dichloromethane. The combined extract was dried (sodium sulfate), filtered and the solvent removed. A quantitative determination was made on this extract by g.l.c. with 5-hydroxymethyl-2-furaldehyde as a standard; yield of **5** 1.7%. The remaining oil was separated by g.l.c. to give **5**, m.p. 23-24°; λ_{max}^{film} 3340s (OH), 2850w, 1655s (C=O), 1540w, 1495s, 1485s, 1390s, 1360s, 1325sh, 1240m, 1200m, 1150s, 1060sh, 1040s, 1020s, 950w, 808s, 790s, 725w cm^{-1}; n.m.r. data in $CDCl_3$: δ 3.87 (singlet, Me-*a*), 4.58 (broad singlet, CH_2 and OH-*b*), 6.18 (1-proton doublet, H-*c*), 6.81 (1-proton doublet, H-*d*), 9.33 (1 proton, H-*e*); *m/e* 139 (100) (M^+), 138 (18) (M^+-H), 124 (12) (M^+-CH_3), 122 (43) (M^+-OH), 110 (14) (M^+-CHO), 94 (10), 93 (11), 82 (17), 67 (18), 57 (30), 55 (38), 42 (32), 39 (36); high-resolution *m/e* 139.0592 (calc. for $C_7H_9NO_2$, 139.0632.

5-Acetoxymethyl-1-methylpyrrole-2-carboxaldehyde (**16**). — To 0.85 g of a crude mixture of compounds **1** and **5** was added 25 ml of chloroform followed by

2 g of fused sodium acetate and 2.5 ml of acetic anhydride. The mixture was kept for 12 h at room temperature. Water (10 ml) was added and after 1 h at room temperature the organic layer was washed with 5 ml of M NaOH and 5 ml of water, dried, and the solvent removed. The resultant mixture was then separated by t.l.c. on 1-mm coated plates. The desired bands were eluted and the product collected. The compound was crystallized from methanol but still contained an impurity. A portion of this material was purified by g.l.c. to give pure **16**, m.p. 20.0–20.2°; λ_{max}^{film} 1745s, 1660s, 1490m, 1478m, 1390m, 1377m, 1347m, 1320m, 1230s, 1155s, 1030s, 1005w, 975m, 955m, 920w, 805s, 790s, 728w cm^{-1}; *m/e* 181 (65), 139 (17), 123 (13.3), 122 (100), 121 (12.8) 94 (17.4), 93 (41), 92 (24.6), 82 (8.7), 67 (13.3), 55 (9.7), 53 (23), 52 (19.5), 51 (13.8), 43 (41.5), 42 (21.5), 41 (17.9), 39 (30.8); high-resolution *m/e* 181.0726, calc. for $C_9H_{11}NO_3$, 181.0738.

2-Acetyl-1-methylpyrrole (**6**). — This compound was synthesized from 2-acetylfuran by the same procedure used for synthesis of **5**. The i.r. and mass spectra were identical with published data[17]. N.m.r. data in $CDCl_3$: δ 2.42 (singlet, Me), 3.93 (singlet, Me), 6.13 (1 proton, two doublets) 6.78 (1-proton apparent singlet), 6.92 (1-proton doublet).

5-Methyl-2-furfuryl acetate (**7**). — To 0.20 g of 5-methylfurfural was added 2 ml of methanol and 0.10 g of sodium borohydride and the mixture was kept for one h at room temperature. An 0.4-ml aliquot of this solution was injected into the g.l.c. apparatus and 5-methyl-2-furfuryl alcohol was separated. To 25 μl of the latter was added 150 μl of acetic acid. After 2 h at room temperature the entire sample was separated by g.l.c., to give **7**, $\lambda_{max}^{CS_2}$ 1740sh, 1710s, 1282s, 1222s, 1165w, 1017s, 965w, 925w, 785s, 750w cm^{-1}; *m/e* 154 (9) 112 (5), 95 (38), 94 (8), 79 (11), 60 (41), 45 (76), 43 (100).

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