*The Degradation of Carbohydrates by Alkali. Part III.** 3-O-*Methyl Derivatives of Glucose and Fructose.*

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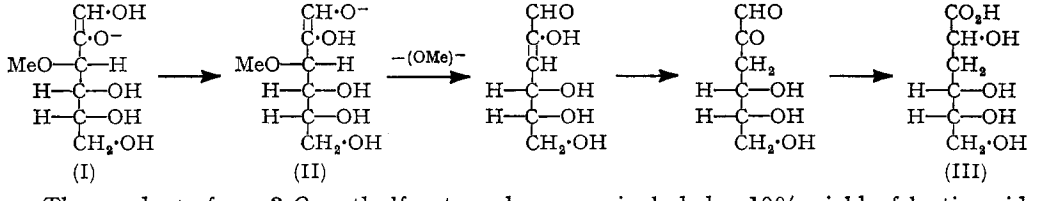
The results of a study of 3-*O*-methyl derivatives of glucose and fructose accord with the view that the site of the etherifying group (when this is in the 3- or the 4-position) regulates the result of their attack by dilute alkali.

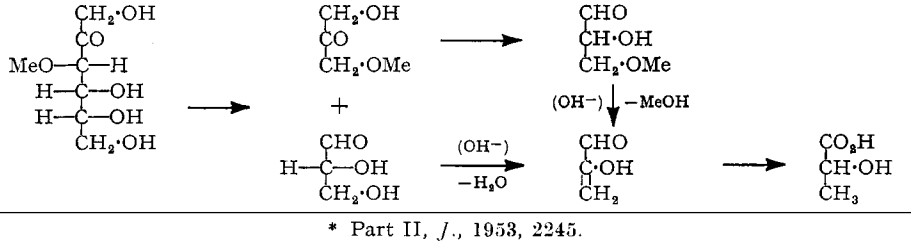
THE general mechanism of the action of dilute alkali on disaccharides has been discussed in Part II * and it appears that it is regulated by the point of attachment of the glycoside grouping (when this is in the 3- or the 4-position) to the parent aldose or ketose. Thus it involves the carbonyl group in the 1-position in 3-glucosidoglucose (laminaribiose) (Corbett, Kenner, and Richards, *Chem. and Ind.*, 1953, 154) but only operates from the 2-position in the cases of 4-glucosido- and 4-galactosido-glucoses (maltose and lactose) after their isomerisation to the corresponding ketoses.

This comparison, as well as the need to explain the behaviour of the monosaccharides towards alkali, impelled us to study that of the 3-*O*-methyl derivatives of glucose and fructose in this respect.

The anion of 3-*O*-methylfructose (I) is in equilibrium with that of the isomeric glucose ion (II) and, as would be expected, either isomeride is rapidly converted in solution into a mixture of the two, of which the relative proportions were determined by means of resorcinol. Consequently each was converted by lime-water at 25° into methyl alcohol and a mixture of Nef's stereoisomeric α- and β-metasaccharinic acids (III) (*Annalen*, 1910, **376**, 1; cf. Bollinger and Prins, *Helv. Chim. Acta*, 1946, **29**, 1061, whose data on 3-deoxymannonic acid seem to indicate its identity with Nef's β-acid):



The products from 3-*O*-methylfructose, however, included a 10% yield of lactic acid, presumably arising according to the scheme:



* Part II, *J.*, 1953, 2245.

The difference between the two cases accords with the usual view that lactic acid formation from glucose or fructose occurs through the ketose rather than the aldose and also with results of experiments on glucose and fructose to be reported later.

The alkyl group then determines the site of reaction, as do the glycosyl groups in the cases cited at the outset, possibly owing to the greater receptivity of the alkaline medium for an alkoxide or glycoside anion than for hydroxyl ion respectively extruded from the carbohydrate anion.

The following Table shows the great speed of the degradation of the methyl derivatives by saturated lime-water at 25°, that of the ketose being the faster. A further Table (p. 282) shows the rate of degradation by 0·05N-aqueous potassium hydroxide to be much slower than that with lime-water, possibly owing to stimulation of carbonyl reactivity by $[CaOH]^+$ comparable with that by lithium ions on the interaction of diazomethane and acetone recorded by Meerwein and Burneleit (*Ber.*, 1928, **61**, 1840).

Time (hr.)	Total hexose (mg.)	Total ketose (mg.)	Total aldose (mg.)	Acid equivs. per mole of hexose	Paper chromatography *		
			3-O-*Methylglucose*.		3-MeG	3-MeF	Sacc. acids
0	340	0	340	0	3	—	—
0·1	340	13	327	0	3	1	—
0·2	340	20	320	0	3	1	—
0·3	340	27	313	0	3	1	—
0·4	340	36	304	0·006	3	1	—
0·5	340	44	296	0·011	3	1	—
0·75	332	53	279	0·031	3	2	1
1·0	319	65	254	0·062	3	2	1
2·0	281	78	196	0·171	2	2	2
2·9	246	75	171	0·271	2	2	2
4·5	206	70	136	0·408	2	2	2
5·5	183	62	121	0·447	2	2	3
7·0	153	54	99	0·543	2	2	3
24	42	3	39	0·872	1	—	3
28	32	2	30	0·886	1	—	3
48	22	1	21	0·914	1	—	3
72	19	—	—	0·935	—	—	—
170	—	—	—	1·003	—	—	—
			3-O-*Methylfructose*.				
0	170	170	0	0	—	3	—
0·1	162	161	1	0·018	—	3	—
0·25	156	154	2	0·046	—	3	—
0·35	148	139	9	0·068	1	3	—
0·45	147	129	18	0·089	1	3	—
0·75	141	116	25	0·157	1	3	1
1·0	131	101	30	0·200	2	3	1
2·0	111	66	45	0·360	2	2	1
3·0	93	47	46	0·474	2	2	2
4·0	86	40	46	0·542	2	2	2
5·0	72	34	38	0·606	2	2	2
6·0	65	26	39	0·662	2	1	2
7·0	59	22	37	0·713	2	1	3
8·0	51	19	32	0·762	2	1	3
24	15	5	10	0·978	1	1	3
30·5	12	4	8	—	1	1	3
48	10	3	7	1·030	1	1	3
54·5	8	—	—	1·050	1	1	3
72	6	—	—	1·054	1	—	3
102	—	—	—	1·062	1	—	3
168	1·5	—	—	1·075	—	—	3

* Numbers denote relative intensity of spots, 3 denoting the greatest. G = glucose, F = fructose, Sacc. = saccharinic.

These results are the more striking in that metasaccharinic acid is not the product of the action of dilute alkali on glucose (cf. Kiliani, *Ber.*, 1882, **15**, 2953), and this will be discussed in a later paper. Meanwhile, it will be noted that the acid is a potential source of 2-deoxy-D-ribose.†

† Patent protection pending.

Experimental

Oxygen-free solutions were obtained as required by saturation with nitrogen.

3-*O*-Methylglucose, from 1 : 2-5 : 6-di*iso*propylideneglucose (Glen, Myers, and Grant, *J.*, 1951, 2568), while having the correct m. p., was shown by chromatography to contain a trace of glucose and was purified by elution from a cellulose powder column (30 × 4 cm. for 2 g.) by aqueous alcohol (98%). The product had m. p. 165—166°, $[\alpha]_D^{20}$ +55·4° (20 hr., *c*, 4·5 in H_2O).

3-*O*-Methylfructose, from 1 : 2-4 : 5-di*iso*propylidenefructose (Glen, Myers, and Grant, *loc. cit.*), after similar treatment to remove traces of fructose, the eluant being the upper layer from a mixture of butanol, ethanol, and water (4 : 1 : 5), diluted with an equal volume of butanol, showed m. p. 125—126°, $[\alpha]_D^{20}$ −53·7° (15 min.; *c*, 1·2 in H_2O). Anderson, Charlton, Haworth, and Nicholson (*J.*, 1929, 1337) reported m. p. 128—130°, $[\alpha]_D^{20}$ −53·5°.

Qualitative Experiments.—(1) A solution of 3-*O*-methylglucose (11·36 g.) in oxygen-free lime-water (3 l.; 0·0412N), after maintenance at 25° for 170 hr. in nitrogen, was stirred with washed Amberlite resin IR-120 (150 g.) until free from calcium ions, and separated from the resin. On distillation the first fraction contained methanol (yield, 66% as estimated from the density of the solution), identified by conversion into the triphenylmethyl ether, m. p. and mixed m. p. 82—83° (cf. Kenner and Richards, *J.*, 1953, 2240). The residual acidic solution, concentrated under reduced pressure at 40° to a syrup, furnished a distillate of pH 5·5 with a trace of volatile acid in the last few drops (pH 2—4). A solution of the syrup in water (100 ml.), after decolorisation by charcoal and neutralisation with calcium carbonate, was again evaporated to dryness. The residual syrup solidified on trituration with ethanol, the crude calcium glucometasaccharinate being finally obtained as a white powder (11·19 g., 96%) which was fractionated as follows. A solution of the crude salt (5·00 g.) in hot water (12 ml.), when kept at 0° for several hours, deposited colourless, cubic crystals of calcium β-glucometasaccharinate (1·18 g.), $[\alpha]_D^{21}$ −22·2° (*c*, 0·4 in H_2O) (Nef, *loc. cit.*, reported $[\alpha]_D^{20}$ −23·25°) [Found: C, 36·5; H, 5·6. Calc. for $(C_6H_{11}O_6)_2Ca$: C, 36·2; H, 5·6%]. Addition of ethanol to the mother-liquors resulted in isolation of the following amorphous white powders: (*a*) from 47% ethanol, 0·77 g., $[\alpha]_D^{21}$ −10·9° (*c*, 0·3 in H_2O), mixed α- and β-forms; (*b*) from 60% ethanol, 1·60 g., $[\alpha]_D^{21}$ −5·2° (*c*, 0·3 in H_2O), shown (see below) to be calcium α-glucometasaccharinate (Found: C, 36·1; H, 5·4%); (*c*) (0·20 g.) and (*d*) (0·32 g.), precipitated from 70% and 82% ethanol respectively, also consisted of α-form.

Strychnine β-*Glucometasaccharinate.*—An aqueous solution (0·1 g. in 10 ml.) of calcium β-glucometasaccharinate was stirred with excess of Amberlite resin IR-120, filtered, and heated on the water-bath for 18 hr. with an excess of powdered strychnine. After cooling to room temperature and filtration from unused strychnine, the solution was evaporated to a syrup from which, after trituration with 92% ethanol, the strychnine salt was obtained as colourlesss needles (0·160 g.). Recrystallised from the same solvent, it sintered and decomposed at 185—190°, and showed $[\alpha]_D^{20}$ −30·5° (*c*, 4 in H_2O) (Found: C, 63·2; H, 6·9; N, 5·4. Calc. for $C_{27}H_{34}O_8N_2$: C, 63·0; H, 6·7; N, 5·4%). Nef (*loc. cit.*) reported decomp. at 180—190° and $[\alpha]_D^{20}$ −30·79°.

" β-*Glucometasaccharin.*" A solution of the strychnine salt (1·05 g.) in water (10 ml.) was treated at room temperature with 0·1N-sodium hydroxide (25 ml.). Strychnine was removed by filtration and by washing of the filtrate with chloroform. After removal of sodium ions by Amberlite resin IR-120, evaporation of the aqueous solution yielded a colourless syrup (0·30 g., 92%) which crystallised after several weeks. Recrystallised from acetone as fine, colourless needles, the " saccharin " showed m. p. 85—88°, $[\alpha_D^{21}]$ +8·16° (*c*, 2 in H_2O) (Found: C, 44·5; H, 6·4. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2%). Nef (*loc. cit.*) reported m. p. 92°, $[\alpha]_D^{20}$ +8·2°. The same product was obtained by removal of calcium ions with Amberlite resin IR-120, from the corresponding calcium salt.

Strychnine α-glucometasaccharinate was obtained from fraction (*b*) by the procedure described in the case of the β-form. Recrystallised from ethanol–ether, the salt sintered and decomposed at *ca.* 185° and showed $[\alpha]_D^{21}$ −19·2° (*c*, 2 in H_2O) (Found: C, 63·3; H, 6·7; N, 5·1%). Nef (*loc. cit.*) reported m. p. 145—147° and $[\alpha]_D^{20}$ −19·5°.

Brucine α-glucometasaccharinate, similarly prepared in aqueous solution, was freed from excess of brucine by extraction with chloroform. The salt sintered and decomposed at 147—150° and showed $[\alpha]_D^{21}$ −22·5° (*c*, 1 in H_2O). Nef (*loc. cit.*) reported m. p. 145—150° and $[\alpha]_D^{20}$ −23·14°.

" α-*Glucometasaccharin.*" Attempted preparation of the lactone by the methods described for the β-isomer yielded a hygroscopic colourless syrup which crystallised partly on trituration

with acetone. The crystals, when drained for several days on a porous plate, showed m. p. (rapid heating) 104—105·5° (Nef, *loc. cit.*, reported m. p. 104°). When heated, even at 60° for prolonged periods, the crystalline material became amorphous and could not subsequently be recrystallised, thus suggesting that some isomerisation to the β-form may have occurred, similar to that described by Nef (*loc. cit.*) in the case of the " galactometasaccharins."

(2) A solution of 3-*O*-methylfructose (2·284 g.) in oxygen-free lime-water (650 ml.; 0·0402N) kept in nitrogen at 25° for 170 hr., when treated according to the procedure of the previous experiment, yielded, after removal and identification of methanol (0·275 g., 73%), a pale yellow syrup which was extracted continuously with ether for 2 days, the extract being kept neutral by the presence of zinc carbonate in water (cf. Evans and Hass, *J. Amer. Chem. Soc.*, 1926, **48**, 2705). From the residual syrup, strychnine β-glucometasaccharinate was readily prepared as described above, showing $[\alpha]_D^{20}$ −30·0° (*c*, 2 in H_2O).

From the ethereal extract zinc lactate trihydrate (0·418 g.) was isolated, and its aqueous solution (10 ml.) was shaken with excess of Amberlite resin IR-120, filtered, neutralised with sodium hydroxide, and heated for 1 hr. with alcoholic 4-bromophenacyl bromide (0·6 g., 20 ml.). Fractional recrystallisation of the product from aqueous ethanol furnished 4-bromophenacyl lactate, m. p. and mixed m. p. 111—113°.

Kinetic Experiments.—The general procedure corresponded to that described in Part II (*loc. cit.*); equivs. of acids formed were determined by back-titration of excess of sulphuric acid in preference to direct titration. Butanol–pyridine–water (3 : 2 : 1·5) was used for chromatography (cf. Jeanes, Wise, and Dimler, *Analyt. Chem.*, 1951, **23**, 415); development was by silver nitrate–sodium hydroxide (Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444), and confirmation of the presence of ketoses by naphtharesorcinol (Hough, Jones, and Wadman, *J.* 1950, 1702); calibration curves for use of the Hagedorn–Jensen method (*Biochem. Z.*, 1923, **135**, 46) for estimating 3-*O*-methylglucose and 3-*O*-methylfructose coincided. The amount of ketose in mixtures of the two was determined by measuring the optical density at 4800 Å resulting when a mixture of ketose solution (5 ml. containing <0·3 mg.), aqueous resorcinol solution (1 ml.; 0·5%), and concentrated hydrochloric acid (5 ml., free from iron) was heated on a boiling-water bath for 8 min. ± 10 sec. (cf. Gray, *Analyst*, 1950, **75**, 314). Calibration showed a linear relation between optical density and concentration up to 0·2 mg./5 ml. and no measurable colour was obtained from a corresponding solution of aldose.

The results thus obtained under oxygen-free conditions from treatment of 3-*O*-methylglucose (0·3400 g.) with lime-water (100 ml.; 0·04055N), and of 3-*O*-methylfructose (0·1700 g.) with lime-water (50 ml.; 0·0395N), each at 25°, have already been cited. In the former case the yield of acid corresponded to formation of metasaccharinic acid only (R_F 0·069 on chromatograms), but in the latter an excess of acid equivalents is observed over those of hexose decomposed. This indicates some conversion of the ketose into lactic acid (cf. Schaffer and Friedemann, *J. Biol. Chem.*, 1930, **86**, 345), and the relative amounts of each acid at any given time

Acid yields from 3-O-*methylfructose in lime-water.*

Time (hr.)	Decomp. (%)	Acid formed (equiv. %)	Lactic acid (%)	Metasaccharinic acid (%)
2·0	34·7	36·0	1·3	33·4
3·0	45·2	47·4	2·2	43·0
5·0	57·7	60·6	2·9	54·8
6·0	61·8	66·2	4·4	57·4
7·0	65·3	71·3	6·0	59·3
8·0	70·0	76·2	6·2	63·8
24	91·1	97·8	6·7	84·4
48	94·0	103·0	9·0	85·0
54·5	95·2	105·0	9·8	85·4
72	96·3	105·4	9·1	87·2
168	99·0	107·5	8·5	90·5

were calculated on the assumption that each molecule of hexose which is decomposed results in the formation either of one equivalent of metasaccharinic acid or of two equivalents of lactic acid.

It thus appears that after 168 hr. (99% decomposition) 8·5% of the ketose had been converted into lactic acid and 90·5% into metasaccharinic acid. This conclusion accords with the findings in the above qualitative experiment. Data in regard to the early stages have been omitted because they are more related to the necessary preliminary formation of intermediate products, as indicated in the introduction.

The final Table reproduces the results of treating 3-*O*-methylglucose (0·4380 g.) with aqueous potassium hydroxide (100 ml.; 0·05N) at 25°.

3-O-*Methylglucose in* 0·05N-*potassium hydroxide.*

Time (hr.)	0·25	0·5	1·0	2·0	4·0	6·0	23·5	26·5
Acid equivs. per mole of hexose	0·009	0·015	0·024	0·044	0·100	0·175	0·522	0·554
Time (hr.)	30·5	58	97	120	144	198	264	435
Acid equivs. per mole of hexose	0·599	0·787	0·882	0·930	0·965	0·99	1·01	1·02

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