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Analysis of Vapourisable Organic Compounds Formed at γ-Irradiation or Pyrolysis of Some Crystal Modifications of Glucose

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Crystalline samples of α-D(+)-glucose, α-D(+)-glucose monohydrate and β-D(+)-glucose have been γ-irradiated (^{60}Co, 1.5 to 12.4 Mrad, preferably 6.2 Mrad), or pyrolysed (175°C, 2h). After addition of water to the samples, the head space vapours over the solutions were analysed using a gas chromatograph at its highest sensitivities (Perkin-Elmer F-6 FID).

The patterns of the chromatograms for irradiated samples vary from those for pyrolysed samples. Smaller but obvious differences are also found when comparing the six chromatograms with one another, an exception being α-glucose and its hydrate which after pyrolyses give the same chromatograms. It has not yet been found possible to identify the small amounts of organic vapours giving the peaks of the chromatograms, nor has the influence of impurities been determined. However, the technique described offers a promising way for further studies in radiation chemistry.

Irradiation or pyrolysis of solid carbohydrates give rise to a great variety of degradation products after dissolution in water.[1–4] Attempts to analyse are hampered greatly by formation of the large number of products, when the carbohydrates have been pyrolysed or irradiated to such a degree that sufficient amounts of an individual degradation product can be detected or isolated. The number of products will be reduced markedly using only the head space vapours over the water solution for analysis, and thus excluding the majority of the compounds not easily vapourized. Since most of the less vapourisable degradation products of carbohydrates are likely of rather polar nature there will be, due to the presence of water, a rather defined step in concentration of the head space vapours between the easily and the less easily vapourisable components. Furthermore, the relatively large amount of water vapour present in the samples injected also appears to reduce the expected interference of the less vapourisable degradation products. Thus, applying

the highest sensitivities of the flame ionisation detector, interpretable chromatograms of the easier vapourisable degradation products are obtained at routine analyses also when low doses of irradiation and low temperatures of pyrolysis have been used.

In the present investigation crystalline samples of α-D(+)-glucose, α-D(+)-glucose monohydrate, and β-D(+)-glucose have been γ-irradiated or pyrolysed. After addition of water the head space vapours have been analysed for presence of organic volatiles. The main interest of this investigation has been to demonstrate that different chromatograms are obtained from the three crystal modifications of glucose and also that irradiation and pyrolysis of carbohydrates give different chromatograms.

EXPERIMENTAL

Chemicals. The carbohydrates used were reagent grades; α-D(+)-glucose (J. T. Baker Co.) and α-D(+)-glucose monohydrate (Hopkins & Williams Ltd.). β-D(+)-glucose was prepared according to Hudson and Dale.[5] The sample obtained had a constant optical rotation $[\alpha]_D^{25}$ of 21.0 (*c*, 2 g/100 ml).

Irradiation. Samples of the carbohydrates were sealed into glass tubes in the presence of air. The ampoules were irradiated at room temperature with ^{60}Co γ-rays at a dose rate of 3.1×10^5 rad/h. After irradiation, the ampoules were opened and the samples were stored in closed glass jars, usually two to six days before analysis.

Preparation of water solutions of irradiated samples. A calculated amount of the irradiated sample was weighed into a thoroughly cleaned ampoule (Johnson and Jergensen Ltd., London), this being used throughout the analytical procedure. The capacity of the lower part of the ampoule, which contained the water solution, was 10.0 ml (i.d. 15 mm). The upper part (i.d. 6.5 mm) containing the head space vapours and helium had a capacity of 3.0 ml. The ampoule was closed tightly with an inert silicone stopper (o.d. 6 mm, "blind hole", Nichols Inc. USA). This stopper was also tight when punctured by an injection needle. With the aid of an injection needle inserted through the stopper, the ampoule was evacuated for half an hour at motor vacuum (0.05 mm Hg). A dry-ice trap prevented back-flush of vapours from the pump into the ampoule. During evacuation, the ampoule was heated to 100°C in an oven of aluminium. At this temperature no head space vapours due to pyrolysis could be detected; *cf.* Table 2. When cooled to room temperature, a calculated volume of degassed water was injected into the evacuated ampoule to give 10.0 ml of a 20 % solution of glucose. The syringe also contained pure helium, thus giving an atmosphere of helium in the ampoule at approximately atmospheric pressure. The ampoule was then immediately transferred to a shaker (Microid shaker, Griffin & George Ltd.), and shaken for a quarter of an hour, *i.e.* to complete dissolution of the solid. Before analysis the ampoule, in upright position, was heated 22 to 24 h at 50°C in an oven of aluminium.

Pyrolysis. A calculated amount of the crystalline modification of glucose was weighed into the ampoule and the evacuation was performed as before. The evacuated ampoules were heated in an oven of aluminium to temperatures between 100 and 200°C for 2 h. At 200°C, the silicone stopper usually loosened due to the high pressure in the ampoule, and thus 175°C was chosen as convenient temperature of pyrolysis. After pyrolysis, water and helium were injected into the cooled ampoule as before. The agitation was done by hand in order to dissolve more rapidly the mass of the ampoule; and then the ampoule was thermostated as given above.

Analytical procedure. The silicone stopper of the thermostated ampoule was punctured simultaneously by the injection needles of two syringes (capacities 10 ml), one syringe containing pure helium, the other empty. The needle of the helium filled syringe was just inserted through the stopper, whereas the other needle almost reached the surface of the water solution. When the piston of the helium-filled syringe was pushed in slowly, the head space vapours of the ampoule (3 ml) together with rinsing helium (7 ml) were transferred to the other syringe, which was immediately used for injection into the gas chroma-

tograph. Prior to use, the syringes were boiled in water, blown with steam, refilled several times with pure nitrogen and helium, and were then kept at 80°C in an oven of aluminium. The syringes used for preparing the water solutions were rinsed in the same way, so also the ampoules. Blanks were run frequently throughout the whole procedure (untreated glucose was used) and with the exception of small peaks of acetone, undisturbed blanks could be obtained (*cf.* Table 2, lower temperatures of pyrolysis).

A gas chromatograph manufactured by Perkin-Elmer, model F-6 FID, in combination with an 1 mV recorder, Speedomax H, was used for this investigation. The highest sensitivities of the chromatograph were used throughout, which was only found possible in combination with carefully bled columns, with stationary phases of silicones or polyethylene glycol (PEG). Since the silicone columns gave a poor separation of the components of the head space vapours, only the PEG column could be used. The column was 2 m long, had an i.d. of 4 mm, and consisted of 20 % PEG 1500 (Mo & Domsjö AB, Sweden) on Chromosorb W. Unfortunately, not all of the columns prepared could be bled to the desired stability. Silanization or acid-washing of the solid support seemed to be without effect in this case. In a paper by Larsson,[6] which has been of guidance in this investigation, the use of all-glass columns has been reported. As a control of our method some of the samples were analysed on an all-glass instrument of the same type as the one used by Larsson. The same chromatograms were obtained as when using aluminium columns and injection port of stainless steel.

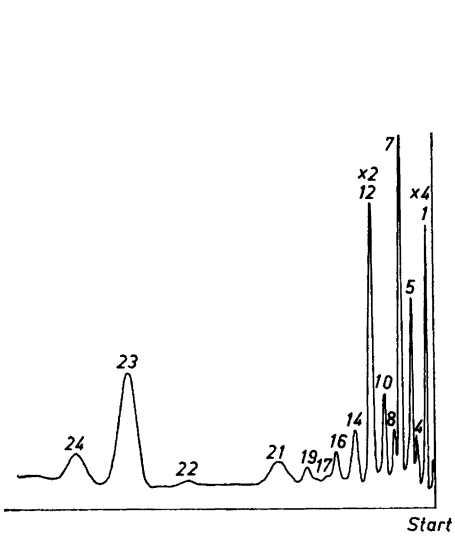

Fig. 1. Gas chromatogram on head space analysis of γ-irradiated α-D(+)-glucose. Dose 6.2 Mrad. The peaks are numbered in sequence of their appearance, *cf.* text and Table 1. Column: 20 % PEG-1500 on Chromosorb W, mesh 60—80, length 2 m, i.d. 4 mm. Chromatograph: Perkin-Elmer F-6 FID; sensitivity 1 unless an other figure is marked above the peak number; temperature 80°C; carrier gas helium 60 ml/min. Recorder: Speedomax H, 1 mV (18 cm), chart speed 30 cm/h.

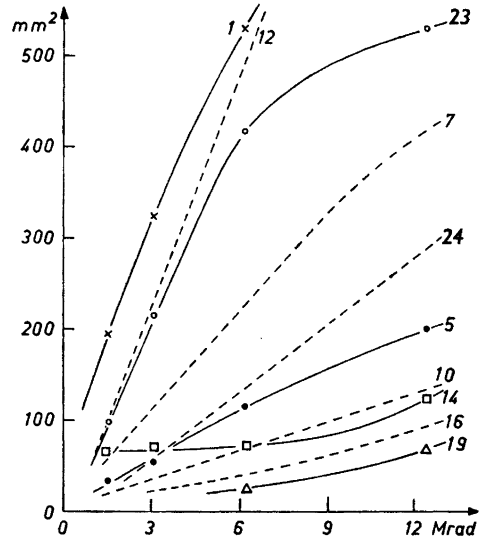

Fig. 2. Dose dependance of some vapourisable compounds, formed by γ-irradiation of α-D(+)-glucose. The response, expressed as integrated area (mm²) of the gas chromatogram recordings, is plotted *versus* dose in Mrad. Numbering of peaks and conditions as given in Table 1 and Fig. 1. Points for dashed curves (peaks Nos. 7, 10, 12, 16, and 23) are not shown.

RESULTS AND DISCUSSION

6.2 Mrad was found to be a sufficient dose of γ-irradiation to give interpretable chromatograms on head space analyses. Even at lower doses, some of the peaks are detectable, which is shown in Fig. 2. However, 6.2 Mrad gives more detailed chromatograms and the components forming the major peaks are still not present in such quantities that they disturb the base line stability on routine analysis (Fig. 1). Higher doses cause overlapping of the peaks in the first part of the chromatograms, and make reading of the chromatograms difficult. It is evident from Fig. 2 that most of the peaks show a linear or nearly linear dose dependence in the dose interval investigated, 1.5 to 12.4 Mrad, for α-D(+)-glucose.

Table 1. Gas chromatography head space analyses of irradiated and pyrolysed samples of D(+)-glucose. Irradiation dose 6.2 Mrad; pyrolysis for 2 h at 175°C. The response is given in mm², obtained by integration of the recordings; conditions as given in text and Fig. 1.

Peak No.	Retention value (H_2O = 1000)	α-Glucose		α-Glucose monohydrate		β-Glucose		Note *
		irradiation	pyrolysis	irradiation	pyrolysis	irradiation	pyrolysis	
1	32	490	190	680	110	5400	>200	
2	56		50		30		25	
3	75		<5		<5		<5	
4	113	30	12	30	20	40	10	(acetaldehyde)
5	140	115		280		645		
6	174		>480		330		300	
7	210	230	45	150	60	165	55	(acetone)
8	246	45		150		250	<5	
9	269		55		25		70	
10	314	70		90		360	<10	
11	351					500	55	
12	393	470		265		320		(methanol, butanone-2)
13	448		20		>1000		>6500	
14	477	70		570		4400		
15	550		27		30		30	
16	623	40				1200		(pentanone-2, and -3)
17	694	<5	5		15		20	
18	767			10				
19	813	25		20		53		
20	913					490		
21	1000	90	75	90	75	**	55	(water)
22	1560	10				510		
23	1940	415		340		55		
24	2270	140		10		60		
25	2690		160		200		300	

* The compounds marked in brackets were found to have similar retention values.
** The peak is partly masked under peak No. 20.

On pyrolysis, only very poor head space chromatograms were obtainable at 150°C and below, whereas at 200°C too high pressure was reached in the ampoule. The conditions used for pyrolysis, *i.e.* 2 h at 175°C, was found to be convenient for all three crystal modifications of glucose.

The chromatograms obtained on the head space analysis of irradiated and pyrolysed samples are collected in Table 1. The peaks of all the six chromatograms are numbered according to their retention values. The peak areas are given in mm^2 since all the chromatograms have been recorded under the same conditions, using the same 1 mV recorder. Since some of the peaks (Nos. 1, 4, 7, and 21) are present in all chromatograms and since the chromatograms of all the pyrolysed samples contain peaks Nos. 1—4, we consider the numbering sufficiently correct to be used at the present stage of the investigation. However, also in those cases where the retention values are exactly the same, there are no proofs for identity. Furthermore, several constituents of the head space vapour might have formed one of the peaks registered. Thus, the peak numbers are given only to facilitate the reading of the chromatograms, and to demonstrate the non-presence of certain peaks.

Table 2. Head space analysis of α-D(+)-glucose pyrolysed for 2 h at different temperatures. The peaks are numbered according to Table 1. The peak areas are expressed in mm^2; conditions, *cf.* Fig. 1.

Peak No.	Retention value (H_2O = 1000)	Temperature of pyrolysis				
		50°	100°	125°	150°	175°
1	32	20		20	20	190
2	56			5	5	50
3	75	20	15	5	5	< 5
4	113		5			10
6	174				5	>480
7	210			10	20	45
9	269	10	10	20	10	55
13	448	5	5			20
15	550					27
25	2690			10	32	160

Comparing the figures of the chromatograms given in Table 1, the marked difference between the composition of the head space vapours of irradiated and pyrolysed samples is very obvious. The peaks Nos. 2, 3, 9, 13, 17, and 25 are present in all the pyrolysed samples, but not in the irradiated ones. The reverse is valid for peaks Nos. 5, 12, 14, 19, 23, and 24. Present in all chromatograms are only the peaks Nos. 1, 4, 7, and 21, of which 21 corresponds to the weak response for water by the flame ionisation detector (FID). These observations favour the assumption that the patterns for formation of the final degradation products are different for pyrolysis and irradiation of crystalline D(+)-glucose. Naturally, the dissimilarity can be caused by different patterns of reactions with impurities present either in the samples of glucose or in the water. This reservation has to be underlined since the degradation

Table 3. Head space analysis of aqueous solutions of some alcohols and ketones. Conditions, *cf.* Fig. 1.

Compound	Concentration in solution % by weight	Peak area on chromatogram mm^2	Relative response mm^2 per ppm in solution
Methanol	1.8×10^{-3}	100	5.5
Ethanol	1.8×10^{-3}	130	7.2
Propanol	1.3×10^{-3}	140	10.8
Butanol	1.8×10^{-3}	330	18.5
Acetone	2.4×10^{-5}	14	57
Butanone-2	2.4×10^{-5}	18	75

products forming the head space vapours can only be a very small part of the whole amount of all the degradation products formed.

The differences for the individual crystalline modifications of glucose are less obvious. However, only irradiation of β-glucose gives the peak number 20 and only irradiation of α-glucose monohydrate gives peak No. 18. Comparing irradiation of α-glucose and its hydrate, peak No. 16 is missing for the hydrate and also peak No. 10 is much smaller here. In case of irradiation of α-glucose and β-glucose, differences are found with several peaks: Nos. 8, 10, 11, 14, 16, and 20. On pyrolysis, the same pair shows differences in peak Nos. 7, 9, 10, 11, and 15. Finally, pyrolysis of α-glucose and its hydrate give the same chromatograms, which seems very reasonable since the hydrate will lose the water of crystallisation at the temperature of pyrolysis used.

It has been desirable to complete this investigation with an estimation of the concentrations of the peak-forming substances in the water-glucose solutions. However, it would then be necessary to identify the peaks of the chromatograms, and this has not yet been found possible. A series of organic compounds have been tested, and some of them have given similar retention values. Since we have only been able to find one type of column (PEG 1500) to give the desired stability and separation, the cases of coincidence can by no means be used as proof for identity. Here we restrict ourselves to report values of head space concentrations of some compounds dissolved in pure water (Table 3). From this table it is clear that the head space vapour is only a very small part of the amount of the compound added to the water. Furthermore, the response recorded is strongly dependent on the vapour pressure and the polarity of the compound tested.

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