CHROM. 3952

The identification of the free carbohydrates present in some grass pollens*

Since grass pollen allergens have been reported variously as carbohydrate[1,2] and protein[3-7], it was considered pertinent to investigate the free sugar content of aqueous extracts of some grass pollens. The free amino acid content of these pollens has been reported previously[8]. Furthermore, although pollen may be in the atmosphere for a considerable time, hay fever sufferers normally associate the severity of their attacks with the time when pollen is shed from the anthers and since at this time the pollen grains are potentially viable, extracts of both viable and non-viable pollens[9] were examined to ascertain whether there was any difference with respect to their free sugar content.

Although numerous sugars[10-16] and sugar alcohols[11,17-22] have been reported in various pollens the only reports on sugars present in grass pollens are given by GOUGH[23] and AUGUSTIN[13,22]. The former found a complex carbohydrate yielding on acid hydrolysis, arabinose, galactose and a non-reducing sugar in *Phleum pratense* L. pollen while the latter reported the presence of glucose, mannose or fructose and inositol in *Dactylis glomerata* L. and *Phleum pratense* L. pollens. No experimental evidence was presented by AUGUSTIN[13] for the resolution of the "mannose–fructose' spot although a claim is made in the discussion that extracts of *P. pratense* and *D. glomerata* pollens contain free glucose, fructose and inositol.

Experimental

The grasses examined and details of their collection and extraction procedure have been reported previously[8]. A summary of the experimental procedures for thin-layer chromatography (TLC) is given in Table I.

TABLE I

SUMMARY OF EXPERIMENTAL PROCEDURES FOR TLC

Adsorbent	Silica gel, buffered with boric acid	Cellulose
Thickness	250 μ	250 μ
Activation	Air dried, 1 h; 110° for 60 min	105° for 10 min.
Solvent system	(I) Methyl ethyl ketone–glacial acetic acid–methanol (3:1:1)	(II) Ethyl acetate–pyridine–water (12:5:4)
Method	Ascending in saturated chamber	
Temperature	20–22°	
Distance	10 cm	
Load	Pollen extracts 2 μl (original extracts diluted 2 in 5 to give a glycerol concentration of 20%) Reference sugars 2 μl (1% solutions of reference compounds in 20% glycerol)	
Detection	Sugars: Anisaldehyde–sulphuric acid[24] Naphthoresorcinol–phosphoric acid[25] Inositol: Silver nitrate and sodium hydroxide solution[26]	

* This work forms part of a thesis submitted by G. H. JOLLIFFE for a Ph.D. degree of the University of London, May, 1967.

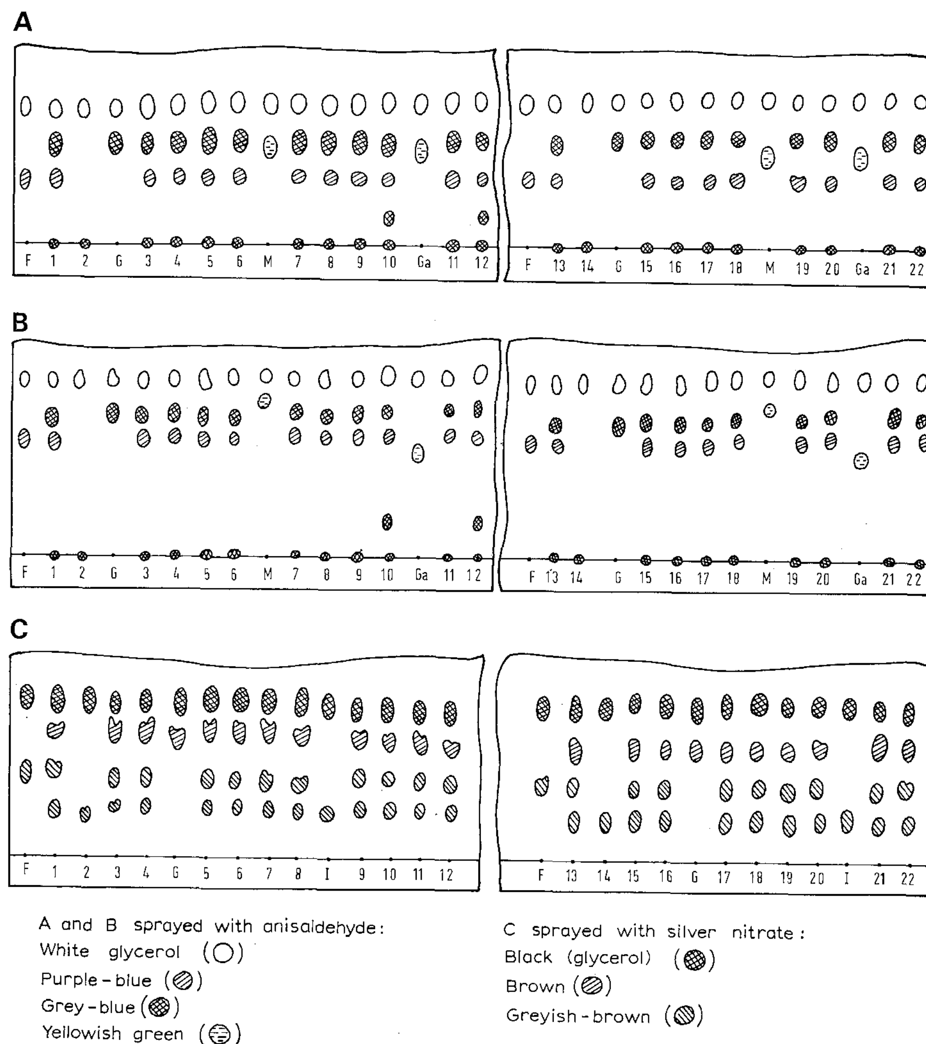

Fig. 1. Tracings of chromatograms. A and C silica gel buffered with boric acid, solvent system I; B cellulose, solvent system II. A and B sprayed with anisaldehyde[24]; C sprayed with silver nitrate[26]. Reference carbohydrates dissolved in 20% glycerol, fructose (F), glucose (G), mannose (M), galactose (Ga), *meso*-inositol (I). Pollen extracts (glycerol concentration 20%): 1, *Anthoxanthum odoratum* L. viable (V), 2, non-viable (N); 3, *Poa trivialis* L. (V), 4, (N); 5, *Dactylis glomerata* L. (V), 6, (N); 7, *Lolium perenne* L. (V), 8, (N); 9, *Alopecurus pratensis* L. (V), 10, (N); 11, *Festuca pratensis* Huds. (V), 12, (N); 13, *Cynosurus cristatus* L. (V); 14, (N); 15, *Arrhenatherum elatius* (L.) J. and C. Presl. (V), 16, (N); 17, *Holcus lanatus* L. (V), 18, (N); 19, *Agrostis tenuis* Sibth (V), 20, (N); 21, *Phleum pratense* L. (V), 22, (N).

Results and discussion

The influence of glycerol on the movement of some simple sugars on silica gel and cellulose thin layers has been reported[27]. Preparation of the reference solutions in 20% glycerol and dilution of the pollen extracts to give a glycerol concentration of 20% were found satisfactory for this work. The results obtained are shown in Table II and Fig. 1.

TABLE II

SUMMARY OF RESULTS OF INVESTIGATION OF POLLEN EXTRACTS FOR FREE SUGARS AND *meso*-INOSITOL

(Layer: Silica gel, buffered with boric acid; Solvent system: Methylethyl ketone–glacial acid–water, 3:1:1)

Pollen extract		*Carbohydrates detected*			
		Fructose	*Glucose*	*Meso-inositol*	*hR_F values of additional components*
Anthoxanthum odoratum L.	V	+	+	+	O
	N	—	—	+	O
Poa trivialis L.	V	+	+	+	O
	N	+	+	+	O
Dactylis glomerata L.	V	+	+	+	O
	N	+	+	+	O
Lolium perenne L.	V	+	+	+	O
	N	+	+	+	O
Alopecurus pratensis L.	V	+	+	+	O
	N	+	+	+	O; 9*
Festuca pratensis Huds.	V	+	+	+	O
	N	+	+	+	O; 9*
Cynosurus cristatus L.	V	+	+	+	O
	N	—	—	+	O
Arrhenatherum elatius	V	+	+	+	O
(L.) J. and C. Presl	N	+	+	+	O
Holcus lanatus L.	V	+	+	+	O
	N	+	+	+	O
Agrostis tenuis Sibth.	V	+	+	+	O
	N	+	+	+	O
Phleum pratense L.	V	+	+	+	O
	N	+	+	+	O

+ = sugar detected; — = sugar not detected.
V = viable pollen extract; N = non-viable pollen extract.
* hR_F of this sugar when chromatographed on a cellulose thin layer using ethyl acetate–pyridine–water (12:5:4) is 11.

Fig. 1 shows that in addition to the uppermost white spot which corresponds with the position of the glycerol reference, the extracts, in general, contain two components and a base-line fraction which yield coloured compounds on spraying.

The rate of movement of the reference galactose, mannose and glucose is similar and while there could be difficulty in distinguishing these sugars by colour after spraying with naphthoresorcinol–phosphoric acid (all give blue colours), galactose and mannose yield a yellow/green colour whereas glucose gives a grey/blue colour when sprayed with the anisaldehyde reagent. There was no evidence of a yellow/green colour in the extracts examined. (Both in rate of movement and in colour after spraying the upper coloured spot corresponds with the reference glucose while the lower coloured spot corresponds with fructose.) Confirmation of the absence of galactose and mannose was obtained from their differential movement on cellulose layers (Fig. 1B). Although AUGUSTIN[13] had difficulty in resolving the "mannose–fructose" spot using paper chromatography, no difficulty was experienced using the thin-layer technique described. Furthermore, AUGUSTIN[13] reported "the lowest spot

appeared in the mannose–fructose position, its colour with naphthoresorcinol (red) placed it in the keto–hexose group". It would, therefore, appear from the facts she presented that the sugar was not mannose but fructose. The former yields a blue colour with naphthoresorcinol whereas the latter gives a red/purple colour[25,28].

All the viable pollen extracts examined showed the presence of fructose, glucose and a base-line fraction (Table II). Variation was found, however, in the extracts from non-viable pollen. Whereas only the base-line fraction was detected in *Anthoxanthum odoratum* and *Cynosurus cristatus*, the *Alopecurus pratensis* and *Festuca pratensis* extracts revealed one additional sugar not present in the viable extracts. This latter sugar gave a blue colour with naphthoresorcinol–phosphoric acid indicating it to be an aldohexose.

The base-line fraction was found to be a non-reducing carbohydrate (Fig. 1C) since it did not reduce the silver nitrate reagent used for the detection of *meso*-inositol. This together with its immobility suggested it may be a polysaccharide. After hydrolysis, with dilute sulphuric acid, of the base-line fraction only glucose (Table II) was detected indicating that the polysaccharide was built up of glucose units.

AUGUSTIN[22] had already shown, by a microbiological assay technique, that *Dactylis glomerata, Alopecurus pratensis* and *Anthoxanthum odoratum* pollens contained free *meso*-inositol. Fig. 1C and the results summarised in Table II confirm her findings and show that this polyhydric alcohol is found in all the grass pollen extracts examined.

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Über die Trennung stereoisomerer 2,4-Dinitrophenylhydrazone parasubstituierter Benzophenone

Unterschiedliche Schmelzpunkt-Angaben bei 2,4-Dinitrophenylhydrazonen von Aldehyden und unsymmetrisch substituierten Ketonen veranlassten THEILACKER[1] zu Untersuchungen über mögliche Ursachen, da angenommen werden konnte, dass die Differenzen nicht immer durch unreine Substanzen hervorgerufen wurden. Es gelang ihm, die zwei stereoisomeren 2,4-Dinitrophenylhydrazone, im folgenden 2,4-DNPH, des *p*-Chlorbenzophenons zu isolieren, die deutliche Schmelzpunktunterschiede zeigten. In diesem Zusammenhang durchgeführte Versuche, das Isomeren-Gemisch durch chromatographische Adsorption an Aluminiumoxid zu trennen, blieben erfolglos. Nachdem EDWARDS[2] durch Mehrfach-Dünnschichtchromatographie mit Phasenumkehr bei 2,4-DNPH aliphatischer Aldehyde die isomeren syn- und anti-Formen trennen konnte und TSCHETTER[3] und REIMANN[4] die isomeren 2,4-DNPH unsymmetrisch substituierter Benzophenone an Kieselgel mit Chloroform bzw. Benzol trennten, gelang uns die Trennung stereoisomerer 2,4-DNPH einiger weiterer monosubstituierter Benzophenone in *para*-Stellung durch Mehrfach-Dünnschichtchromatographie.

Experimentelles

Für die Versuche standen Benzophenone zur Verfügung, die an einem Phenylkern in *para*-Stellung Fluor, Chlor bzw. Brom oder eine Hydroxyl-, Methyl- bzw. Äthyl-Gruppe als Substituenten enthielten. Durch Umsetzen von jeweils 0.5 g der Ketone mit 0.8 g 2,4-Dinitrophenylhydrazin in 50 ml siedendem Äthanol unter Zusatz von 4 ml konzentrierter Schwefelsäure sind die 2,4-DNPH-Derivate hergestellt worden.

Zur Sicherung der Ergebnisse sind als Modellsubstanzen die stereoisomeren 2,4-DNPH des *p*-Chlor-benzophenons nach der von THEILACKER[1] beschriebenen Arbeitsweise aus dem Reaktionsgemisch isoliert und als Vergleichssubstanzen einzeln und im Gemisch chromatographiert worden. Ausserdem sind durch präparative Dünnschichtchromatographie diese Stereoisomeren getrennt, aus der Schicht mit Chloroform eluiert und nach Umkristallisieren aus Chloroform durch Schmelzpunkt-Bestimmung charakterisiert worden.