Journal of Chromatography, 85 (1973) 171–173

CHROM. 6883

Note

The separation and identification of fatty acids from *Macrophomina phaseoli* by thin-layer and gas–liquid chromatography

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(Received May 2nd, 1973)

Knox-Davies[1] showed that groundnut oil, in the presence of ultraviolet light, induced sporulation in *Macrophomina phaseoli*. We identified the lipid responsible as oleic acid[2]. It was therefore necessary to determine the fatty acid composition of *M. phaseoli* in order to be able to evaluate the metabolic significance of oleic acid in photosporogenesis.

The fatty acid composition of *M. phaseoli* was complex. In order to avoid incorrect designation of some of the fatty acids, the extracted fatty acids were separated by argentation thin-layer chromatography (TLC) into fractions with different degrees of unsaturation. The composition of each fraction was then determined by means of gas–liquid chromatography (GLC). The individual fatty acids were identified by using the relative retention times of standard fatty acids together with the equivalent chain-length (ECL) table of Hofstetter *et al.*[3] for rare and known fatty acids.

Cultures of *M. phaseoli* were grown on asparagine–glucose agar[4] and allowed to overgrow inserted paper segments which were placed on top of the culture media. The segments, when coated with mycelial growth, were extracted with chloroform–methanol (2:1) according to the method of Folch *et al.*[5]. The extracted residue was saponified and the unsaponifiables were extracted with light petroleum (boiling range 30–40°) and discarded. The fatty acid salts were acidified and extracted with light petroleum (boiling range 30–40°), evaporated to dryness under a stream of nitrogen and then esterified with anhydrous methanol containing 12% of BF_3[6].

Argentation–TLC plates were prepared according to the method of Wood and Snyders[7]. The developing solvent system was 15% diethyl ether in light petroleum (boiling range 30–40°). The developed plates were sprayed with rhodamine–dichlorofluorescein reagent[8], and the different lipid areas were rendered visible under ultraviolet light and marked with the aid of a needle. The different fatty acid methyl ester bands were scraped off the plates and the powder was extracted with diethyl ether. The ethereal extract of each band was filtered through a sintered-glass funnel, dried over Na_2SO_4 and evaporated to dryness under a stream of nitrogen. The residue was dissolved in CS_2 and analyzed on a 20% diethylene glycol succinate GLC

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TABLE I

RETENTION DATA FOR SATURATED, MONO-, DI- AND POLY-UNSATURATED FATTY ACID METHYL ESTERS OF *M. phaseoli*

Methyl esters	*Retention time (min)*	*Relative retention time*		*Methyl esters*	*Retention time (min)*	*Relative retention time*	
		Relative to $C_{18:0}$	*Relative to* $C_{18:1}$			*Relative to* $C_{18:0}$	*Relative to* $C_{18:1}$
Saturated fatty acids				*Mono-unsaturated acids*			
Unknown	0.67	0.130	0.12	Palmitoleic ($C_{16:1}$)	3.5	0.685	0.604
Unknown	0.85	0.164	0.147	Heptadecenoic ($C_{17:1}$)	4.5	0.880	0.776
Unknown	0.96	0.185	0.166	Oleic ($C_{18:1}$)	5.8	1.140	1.000
Lauric ($C_{12:0}$)	1.17	0.230	0.201	Eicosenoic ($C_{20:1}$)	9.9	1.940	1.706
Unknown	1.30	0.254	0.224	Unknown	11.5	2.260	1.978
Unknown	1.55	0.304	0.267				
Myristic ($C_{14:0}$)	1.85	0.363	0.319	*Di-unsaturated acids*			
Pentadecylic ($C_{15:0}$)	2.35	0.460	0.405	Unknown	1.78	0.350	0.307
Palmitic ($C_{16:0}$)	3.02	0.588	0.520	Unknown	2.68	0.525	0.462
Margaric ($C_{17:0}$)	3.90	0.765	0.673	9,12-Pentadienoic ($C_{15:2}$)	3.78	0.741	0.651
Stearic ($C_{18:0}$)	5.10	1.000	0.880	Linoleic ($C_{18:2}$)	7.28	1.428	1.260
Arachidic ($C_{20:0}$)	8.85	1.735	1.530	11,14-Octadecadienoic ($C_{18:2}$)	8.40	1.650	1.450
Heneicosanoic ($C_{21:0}$)	10.50	2.060	1.810	Eicosadienoic ($C_{20:2}$)	12.40	2.435	2.140
Behenic ($C_{22:0}$)	15.50	3.400	2.670	Unknown	18.60	3.650	3.200
Unknown	21.00	4.12	3.720				
Unknown	27.50	5.39	4.750	*Poly-unsaturated acids*			
				α-Linolenic ($C_{18:3}$)	9.25	1.815	1.595

column at a temperature of 198° and a nitrogen flow-rate of 50 ml/min. The results are given in Table I.

No difference in the fatty acid composition was observed between cultures of *M. phaseoli* grown in the dark or under ultraviolet irradiation.

The necessity for oleic acid to be obtained from external sources for participation in morphogenic changes of photosporogenesis therefore seems somewhat uncertain.

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