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SURFACE LIPIDS OF WHEAT STRIPE RUST UREDOSPORES, *PUCCINIA STRIIFORMIS*, COMPARED TO THOSE OF THE HOST

LARRY L. JACKSON, LARRY DOBBS, ARLINDA HILDEBRAND and RITA A. YOKIEL
Department of Chemistry, Montana State University, Bozeman, MT 59715, U.S.A.

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Abstract—A surface lipid extract was made of uredospores of wheat stripe rust, *Puccinis striiformis*. The major components of the extract are β-diketones, *n*-alcohols and hydrocarbons. The surface lipid extract of the host wheat has a composition that is qualitatively similar, if not the same, in the major components. Even though there are quantitative differences in the two extracts, it appears that at least the three major components appear on the uredospore surface as a result of the host-parasite relationship.

INTRODUCTION

THE COMPOSITION of the surface lipids of only a small number of fungal spores has been studied.[1] Likewise, the biological role(s) that the surface lipids play is not known, but they may be involved in prevention of desiccation, in providing a barrier to substances entering the spores, and in the germination and infection process.[1]

In 1965, Baker and Strobel[2] first reported on the surface lipids of the uredospores of wheat stripe rust, *Puccinia striiformis*. Their analysis indicated the presence of hydrocarbons, wax esters, triglycerides, diglycerides, free fatty acids, alcohols and other unidentified materials. The hydrocarbons were the only fraction studied to any extent and they were characterized as a paraffin series from C_{19} to C_{31} with major components of C_{29}, C_{31}, C_{27}.

The first observation of similarities between fungus and host surface lipids was observed in three species of *Tilletia* by Laseter *et al.*[3] The hydrocarbon contents of the three *Tilletia* species, all grown on the same variety of wheat, were identical and also the same as the hydrocarbons of the wheat. On the other hand, Laseter *et al.*[4] found the relative alkane distributions for *Ustilago maydis* chlamydospores different in infected and uninfected corn kernels.

In our efforts to extend the results of Baker and Strobel[2] on *P. striiformis*, we observed the presence of β-diketone in the surface lipid extract. Tulloch and Weenink[5] had previously observed β-diketone in wheat surface lipids and we had observed β-diketones in barley surface lipids.[6] In the light of this information and the possibility that there might be a host-fungus relationship in the surface lipids, we were prompted to investigate the surface lipids of both *P. striiformis* uredospores and of the host plant.

[1] WEETE, J. D. (1972) *Phytochemistry* **11**, 1201.
[2] BAKER, K. and STROBEL, G. A. (1965) *Proc. Mont. Acad. Sci.* **25**, 83.
[3] LASETER, J. L., HESS, W. M., WEETE, J. D., STOCKS, D. L. and WEBER, D. J. (1968) *Can. J. Microbiol.* **14**, 1149.
[4] LASETER, J. L., ORO', J. and WEBER, D. J. (1966) *Phytopath.* **56**, 886.
[5] TULLOCH, A. P. and WEENINK, R. O. (1969) *Can. J. Chem.* **47**, 3119.
[6] JACKSON, L. L. (1971) *Phytochemistry* **10**, 487.

RESULTS

The hexane extract of *P. striiformis* uredospores amounted to 1·6 ± 0·2% of the spore weight and the subsequent chloroform extract only extracted an additional 0·3 ± 0·1%. This is in reasonable agreement with other uredospore extractions at 1–5%.[7,8] The significantly smaller amount extracted with chloroform also indicates that the majority of the surface lipid was extracted by the hexane. The extracts were free of orange pigment which also indicates that internal carotenes were not extracted using this procedure.

The hydrocarbon fraction amounted to 14·6 ± 2·4% of the surface lipid extract, with better than 90% of the hydrocarbons being normal alkanes. There appeared to be some material in the hydrocarbon fraction that was not absorbed by molecular seive 5A and that did not allow the gas chromatogram to return to base line between *n*-alkane peaks. This was also observed in the chromatograms of *P. striiformis* hydrocarbons by Baker and Strobel,[2] and Weete.[9] The composition of the *n*-alkanes is shown in Table 1. The major *n*-alkanes were C_{29}, C_{31}, and C_{27} in decreasing quantities. This is in line with what has been previously reported;[1,2] however, the quantitation is different and this will be discussed later.

TABLE 1. COMPOSITION OF SURFACE LIPID EXTRACT FRACTIONS FROM UREDOSPORES OF *Puccinia striiformis* AND IT'S HOST LEMHI WHEAT

	Component % in each fraction										
	Wheat		Uredospore		Wheat	Uredospore		Wheat		Uredospore	
Carbon chain No.	Hydro-carbon	Hydro-carbon	Alcohols	Free fatty acids	Free fatty acids	Triglyceride A fatty acids	Triglyceride B fatty acids	Wax esters Fatty acids	Wax esters Alcohols	Wax esters Fatty acids	Wax esters Alcohols
12					2	1	3				
14				5	1	28	86	tr		1	
16				20	12	26	10	25	8	15	tr
16:1						2					
18				5	4	8	1	12	9	19	2
18:1				4		13		7			
18:2						5					
18:3					57	8					
20				26	4	3		30	9	26	5
21		tr		1							1
22		r		38	6	5		24	28	24	11
23	tr	1									
24	tr	1	3	1	7	1		3	30	6	17
25	2	5									
26	1	2	7		2				5		8
27	8	13									
28	1	2	86		4				12		50
29	47	43									
30	tr	2	4								2
31	34	25									
32	tr	1	tr								
33	6	4									

tr = less than 0·6 but greater than 0·1%.

The major fraction (33 ± 5%) of the surface lipid extract is β-diketonic. The composition, however, is very simple in that greater than 97% is hentriacontan-14,16-dione with an observable amount (less than 3%) of heptacosandione. To our knowledge this is the first report of β-diketones associated with fungal surface lipids.

[7] DALY, J. M., KNOCHE, H. W. and WIESE, M. V. (1967) *Plant Physiol.* **42,** 1633.
[8] WOODBURY, W. and STAHMANN, M. A. (1970) *Can. J. Botany* **48,** 499.
[9] Personal communication.

Another major fraction is the long chain alcohols which amount to 21 ± 3% of the surface lipid extract. All are normal saturated long chain alcohols with octacosanol at greater than 80% (Table 1).

Free fatty acids only amount to 9·0 ± 1% of the surface lipid extract. In Table 1, however, it can be seen that the principal fatty acids are saturated with long chain (C_{22} and C_{20}) fatty acids predominating. The only unsaturated fatty acid is monounsaturated octadecenoic acid.

The triglycerides make up about 10 ± 3% of the surface lipid extract and since the quantity varies considerably there may be some contaminating internal lipid. The triglycerides can be separated by TLC into fraction A (upper band) and fraction B (the lower band). The fatty acid composition of the two fractions are shown in Table 1. Fraction B is interesting since it contains predominately myristic acid and contains no unsaturated fatty acids or fatty acids longer than 18 carbons. Overall the triglyceride fatty acid composition is quite different from the free fatty acid composition in that the triglycerides have more unsaturated fatty acids and more shorter chain 14 and 16 carbon fatty acids. Although Tulloch and Ledingham[10] have reported *cis*-9,10-epoxyoctadecanoic acid in the total uredospore lipid of other *Puccinia* species, we were unable to detect epoxy or dihydroxy acids in the surface lipid extracts of *P. striiformis*. Baker and Strobel[2] discuss the possibility that a component they obtained with an R_f of slightly less than a wax ester might be an ester of an epoxy acid; this now turns out to be β-diketone.

Wax esters and sterol esters make up 6·2 ± 0·6% of the surface lipid extract, nearly 95% of the esters being of the wax ester type. The fatty acid and alcohol components of the ester fractions are shown in Table 1. The fatty acids of the sterol ester fraction are composed of 14:0 (3%), 16:0 (11%), 18:0 (85%) and 20:0 (1%). The sterols were tentatively identified by GLC as cholesterol (9%), sitosterol (47%); there was also an unknown sterol (44%) with a longer retention time than sitosterol which is unlikely to be of the ergosterol type previously observed in rust fungi.[11] Due to insufficient sample, the structure was not determined.

The remaining 5% of the surface lipid extract was not completely characterized due to insufficient sample; however, minor quantities of diglycerides, 31 carbon hydroxy-β-diketones, as well as 30 and 32 carbon ketones were observed.

A cursory examination of the host (Lemhi wheat) surface lipids (Table 1) revealed that the principal components were normal alcohols (65%), β-diketones (16%), hydrocarbons (10%), free fatty acids (5%) and wax esters (2%). The composition of the β-diketone and alcohol fractions were qualitatively and quantitatively the same as the corresponding wheat stripe rust surface lipid fractions (Table 1). The free fatty acid composition was different from any fractions from the wheat stripe rust and is shown in Table 1. About 2% of the wheat surface lipid extract were wax esters and the alcohol and acid composition is shown in Table 1.

Lemhi wheat surface lipids are qualitatively similar to Little Club wheat,[5] but there are quantitative differences. This is not unusual since Little Club wheat was chosen by Tulloch and Weenink[5] because most of the components could be isolated in reasonable yield, rather than because the surface lipid contained major portions of just one or two components.

[10] Tulloch, A. P. and Ledingham, G. A. (1962) *Can. J. Microbiol.* **8**, 379.
[11] Jackson, L. L. and Frear, D. S. (1968) *Phytochemistry* **7**, 651.

DISCUSSION

The qualitative similarities between the surface lipids of *P. striiformis* uredospores and the host are predominantly in the β-diketone, alcohol and hydrocarbon fractions. The hydrocarbon content of both extracts are similar on a percentage basis (10–15%), but there is some additional material in the uredospore. The quantitative similarities in the two hydrocarbon fractions are apparent in Table 1. The differences might be due to a contaminant in the uredospore hydrocarbons making that fraction difficult to separate and quantitate by GLC or to independent synthesis of hydrocarbons by the uredospores. Evidence for the last explanation is found in the presence of long chain fatty acids (greater than 18 carbons) in the free fatty acid fraction of the uredospores (Table 1) which might be intermediates in an elongation-decarboxylation synthesis of hydrocarbons.[12] The free fatty acids may or may not arise from the host surface lipids, since the host has all of the observed fatty acids but in addition has a major portion of its free fatty acids as 18:3 absent from the uredospores.

The uredospore hydrocarbon composition reported here differs quantitatively from that reported previously;[1,2] however, in the previous papers the host wheat was not mentioned and there seems to be varietal differences in hydrocarbon compositions. Thus, the hydrocarbon composition of Little Club Wheat[5] is different quantitatively from Lemhi wheat.

Comparison of the β-diketone and free alcohol composition indicates that they are produced by the host and transferred to the uredospore. The difference in amounts can only be speculated on, with the information available at present. It would appear that when the spore is formed and prior to its release it is exposed to more β-diketone than is present on the surface of the wheat leaf. Possibly infected wheat leaves synthesize more β-diketone than uninfected wheat leaves and thereby make more β-diketones available to the emerging uredospores. Genetic information on different wheat lines indicates that suppression of β-diketone formation is correlated with the appearance of normal primary alcohols.[13] Thus with *P. striiformis* infection there may be a suppression of primary alcohol formation and appearance of additional β-diketone.

The surface lipids of rust uredospores may be involved in the germination and infection process.[8,14] β-Diketones may have an active role here but this has yet to be tested.

EXPERIMENTAL

Uredospores of *P. striiformis* were collected from a field plot of Lemhi wheat near Bozeman, Montana, in mid July to the first week of August of each year collections were made. Collections were made with a vacuum cleaner employing paper collecting bags. The larger debri was removed from the uredospores by sieving through an 80-mesh brass sieve. The uredospores were not stored but extracted immediately. Samples of leaves of uninfected Lemhi wheat were taken from the same plot at the same time that spores were being taken. Leaves were classified as uninfected if no uredia were present even though some showed chlorotic areas.

Extraction. The uredospores were separated into three aliquots, weighed and extracted by suspension in 2 ml hexane per g of uredospores for 15 min. The spores were separated from the solvent by vacuum filtration on fritted glass, and reextracted with $CHCl_3$ using the same technique. The leaves were air dried for 24 hr, weighed, clamped at the cut end with a metal clip and repeatedly dipped in hexane for 3 min. The extraction procedure was then repeated with $CHCl_3$. The extracts were independently evaporated to near dryness under vacuum, transferred to preweighed vials, evaporated to dryness N_2 and weighed.

Chromatography. Preparative and analytical TLC was carried out on silicic acid (Adsorbosil 3) coated plates developed in hexane only for hydrocarbons, hexane–toluene (9: 1) for wax and sterol esters, hexane–Et_2O–HOAc (85:15:1) for the remainder of the lipids. GLC of hydrocarbons, β-diketones, alcohols, wax

[12] KOLATTUKUDY, P. E., BUCKNER, J. S. and BROWN, L. (1972) *Biochem. Biophys. Res. Commun.* **47**, 1306.
[13] BARBER, H. N. and NETTING, A. G. (1968) *Phytochemistry* **7**, 2089.
[14] ALLEN, P. J. and DUNKLE, L. D. (1971) *Morphol. Biochem. Events Plant-Parasite Interactions, Proc. Conf.* 1970, p. 23, Phytopath Soc. Japan, Tokyo.

esters, sterols and fatty acid methyl esters was carried out on a 1% SE-30 on Gas Chrom Q. column (2 mm × 2 m) programmed from 150 to 300° over 15 min. Unsaturated fatty acid methyl esters were characterized on a 15% EGS on Gas Chrom P column (4 mm × 2 m) isothermal at 170°. All GLC traces were compared to standards by running sample, then standard, and then mixed sample and standard.

Characterization. Hydrocarbons, alcohols and β-diketones were characterized by GLC, TLC, IR, NMR, UV and chemical techniques reported previously.[6] Triglycerides and wax esters were transesterified using the technique of Morgan *et al.*,[15] and free fatty acids were methylated with CH_2N_2.[16]

Quantitation. Quantitation was made by weight of preparative TLC fractions, and disc integration of homolog on GLCs using a FID. Each chromatogram was repeated (3×) and the results averaged.

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