LIPIDIC COMPOUNDS FROM THE EXTRACT OF THE SPRINGTAIL *Tetrodontophora bielanensis* (WAGA)

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Surface and internal lipidic compounds from the springtail *Tetrodontophora bielanensis* (WAGA) were investigated. Using chromatographic and spectroscopic methods the following compounds were detected: n-alkanes ($C_{15}-C_{35}$), lycopane ($C_{40}H_{82}$), olefin $C_{40}H_{80}$, seven groups of esters (including sterol esters), secondary alcohol $C_{40}H_{82}O$, triacyl glycerols, free saturated and unsaturated fatty acids ($C_{12}-C_{22}$) and free sterols (cholesterol and desmosterol).

Springtails represent a group of insects which is phylogenetically and anatomically very primitive. In connection with a broader study of natural lipidic compounds we carried out a detailed analysis of extractive compounds from the springtail *Tetrodontophora bielanensis* (WAGA) (Insecta: *Collembola*)*. Nothing is yet known on the composition of its lipidic components. In literature only studies with biochemical orientation have been published[1-3] so far. Further papers concerning other species of the orders of *Collembola* deal with the composition of the proteins from cuticle[4], properties of the wax layer with reference to the antiwetting properties[5] and to the water balance[6], and with the participation of *Collembola* in the decomposition of crop residues[7].

EXPERIMENTAL

Experimental material (animals) was collected in the Giant Mountains (Northern Bohemia) in the summer of 1982 when an accumulation of animals on the surface of pools took place in consequence of frequent rains.

Extraction. The live material (43 g, 7300 animals), freed of impurities, was introduced into a glass column provided with a fritted bottom and eluted gradually with n-pentane (a total of 200 ml). After evaporation of the solvent an extract was obtained (extract A, 200 mg). The remaining material was further extracted in a separating funnel with a chloroform–ethanol mixture (1 : 1, a total of 600 ml). After combination of the extracts the lower layer was filtered, dried over $MgSO_4$ and evaporated, to yield extract B (1·50 g). Both extracts were analysed separately using the same procedure.

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Column chromatography. The extract B (1·50 g) was adsorbed on 9 g of inactive silica gel and chromatographed on a silica gel column (3 × 36 cm, 150 g, 0·06–0·12 mm, 12·5% water content) using light petroleum (b.p. 40–60°C) as eluent and applying an increasing linear gradient of diethyl ether (0–100%). The composition of the fractions was monitored by means of thin-layer chromatography (TLC). The fractions, or intermediary fractions, which did not give a single spot on TLC were further separated by preparative TLC on silica gel.

Extract A was chromatographed in an analogous manner. The results of the analysis are presented in Table I

Transesterification[8]. A sample (esters, triacyl glycerols or acids – 1 mg) was heated in a sealed ampoule with 0·05 ml of tetrachloromethane and 0·05 ml of methanol (previously saturated with 5% of gaseous hydrogen chloride) at 70°C for 1 h. The mixture was neutralized with gaseous

TABLE I

Composition of Extracts A and B

Compound	A		B	
	yield mg	%	yield mg	%
saturated hydrocarbons	2·8	1·4	3·1	0·2
unsaturated hydrocarbon $C_{40}H_{80}$			0·8	0·1
intermediary fractions	1·0	0·5	2·1	0·1
esters 1	4·4	2·2	2·8	0·2
esters 2	4·7	2·4	11·3	0·8
esters 3	—	—	10·8	0·7
esters 4	2·0	1·0	—	—
secondary alcohol $C_{40}H_{82}O$	—	—	2·0	0·1
esters 5	—	—	2·9	0·2
triacylglycerols	14·0	7·0	465·5	31·0
intermediary fractions	3·4	1·7	15·8	1·1
fatty acids	34·9	17·4	180·1	12·0
intermediary fractions	6·3	3·2	14·7	1·0
sterols	4·0	2·0	26·6	1·8
esters 6	8·0	4·0	1·5	0·1
esters 7			1·5	0·1
heterocyclic nitrogen-containing compound $C_{11}H_{13}N_3O$	12·7	6·3	25·3	1·7
heterocyclic nitrogen-containing compound $C_9H_9N_3O_2$	10·3	5·2	81·0	5·4
unknown	—	—	25·0	1·6
held on column and lost at TLC[a]	91·5	45·7	627·2	41·8
Total	200	100	1 500	100

[a] Calculated from difference.

ammonia. After centrifugation of the precipitate the supernatant was injected directly into the gas chromatograph.

Silyl derivatives. A sample (glycerol, alcohol, methyl esters of hydroxy acids — 1 mg) was heated in a sealed ampoule with 0·05 ml of acetonitrile and 0·05 ml of N,O-bis(trimethylsilyl)-trifluoroacetamide containing 1% of trimethylchlorosilane (REGISIL) at 120°C for 1 h. The reaction mixture was injected into the gas chromatograph.

Acetates. A sample (sterols — 1 mg) was dissolved in 0·05 ml of a 2 : 1 mixture of pyridine and acetic anhydride and allowed to stand at room temperature overnight. The mixture was evaporated in a vacuum, diluted with 0·05 ml of $CHCl_3$ and injected into the gas chromatograph.

Hydroxylation of unsaturated acids. A sample (methyl esters of unsaturated acids, obtained from the fraction of triacyl glycerols and the fraction of free acids — 6 mg) was processed by a method described earlier[9–13]. The product was analysed by means of GC-MS.

Gas chromatography (GLC) was carried out on a Packard 427 chromatograph with flame ionization detector, a glass injection system and glass columns (0·24 × 185 cm). The packing of the column consisted of 3% OV-101 on Gas-Chrom Q (100–120 mesh), 3% OV-17 on Gas-Chrom Q (100–120 mesh) and 5% SILAR-10C on Gas-Chrom Q (80–100 mesh) (Applied Science Laboratory, U.S.A.). The Kováts retention indices[14] *I* are given in Table II.

Mass spectrometry (MS) was carried out on an AEI MS 902 spectrometer (Associated Electric Industries, Manchester, G. B.) connected with a Pye Series 104 (Model 64) gas chromatograph. The samples of individual products were introduced into the ion source *via* the direct inlet at 100–140°C. Mixtures were injected into the gas chromatograph coupled to the mass spectrometer by means of a heated metal capillary (0·3 mm i.d., 80 cm length) and a Watson–Biemann separator. The mass spectrometer operated at electron beam energy of 70 eV and full accelerating voltage of 8 kV. The mass spectra were recorded at a resolving power 1 000, high resolution measurements were performed at a resolving power adjusted to 10 000.

IR spectroscopy was carried out on a UR-20 instrument (Carl Zeiss, Jena) in 0·01 cm cells (5% solutions in CCl_4), or on a Perkin–Elmer 621 instrument, in KBr micro pellets.

TABLE II

Kováts retention indices *I*[a]

Compound	Temperature °C	OV-101 *I*	OV-101 SD	OV-17 *I*	OV-17 SD
2,6,10,14,19,23,27,31-Octamethyl-dotriacontane (lycopane, $C_{40}H_{82}$)	280	3 509·3	± 0·5	3 445·1	± 0·7
Unsaturated hydrocarbon ($C_{40}H_{80}$)	280	3 535·5	± 0·4	3 539·4	± 0·4
Secondary alcohol ($C_{40}H_{82}O$)	280	3 708·9	± 0·4	3 728·8	± 0·8
Heterocyclic nitrogen-containing compound ($C_{11}H_{13}N_3O$)	170	1 671·2	± 1·9	1 984·9	± 0·2
Heterocyclic nitrogen-containing compound ($C_9H_9N_3O_2$)	170	1 645·1	± 1·2	2 022·2	± 0·2

[a] Mean values of *I* were calculated from at least 5 measurements; the error is expressed as an estimate of standard deviation.

RESULTS AND DISCUSSION

Saturated hydrocarbons. According to GLC analysis both extracts contain several types of hydrocarbons. To a lesser extent they contain the homologous series of n-alkanes (C_{15}—C_{35}), *i.e.* 17% of the total amount of hydrocarbons in extract A, and 7% of hydrocarbons in extract B, without predominance of the odd members. The rest is a further homologous series, C_{40}—C_{51}. The first and most abundant homologue (56% in extract A, 40% in extract B) was identified by means of mass spectrometry as 2,6,10,14,19,23,27,31-octamethyldotriacontane (lycopane, $C_{40}H_{82}$). This isoprenoid hydrocarbon has not yet been described either in present day flora or fauna. It has been found only in shale oils[15–17], crude oils[18,19] and marine sediments[20,21], and it has also been prepared several times synthetically[22–26]. As regards other homologues, it may be considered on the basis of gas chromatogram records, that they are not individual, but still mixtures of isomers.

The unsaturated hydrocarbon was present in the hydrocarbon fraction of both extracts. From extract B it was isolated by means of preparative TLC on silica gel impregnated with silver nitrate, the yield was 0·8 mg. According to GLC and MS analysis it is an individual compound of mol. weight 560 ($C_{40}H_{80}$) of non-isoprenoid nature, with a branched chain ($C_{20}H_{41}$—CH(CH_3)—$C_{18}H_{35}$). In an attempt at the determination of the double bond by means of hydroxylation with OsO_4 and subsequent silylation[9–13] the molecule was degraded (as determinated by GLC).

Esters. A survey of the isolated groups of esters, classified according to decreasing R_F values, measured on a thin layer of silica gel, is given in Table III. Individual groups of esters were gas chromatographed both in the original state (only esters 3 gave peaks) and after reesterification[8]; in all groups of esters octadecenoid acid ($C_{18:1}$) was the dominant acid.

Esters 1 are interesting owing to their unusually high R_F values. In contrast to all subsequent esters it was practically impossible to reesterify esters 1 completely by the commonly used procedure; only traces of acids were detected in this manner (see Table III) and an alcoholic component with a relative retention time (RRT) 3·6 (in GLC on the OV-101 phase).

Esters 2. After reesterification alcohols with a higher molecular weight than usual in the so far known sterols and triterpenic alcohols[27] (RRT 3·2, 3·6 and 4·3) were found in addition to cholesterol and several further unidentified sterols. Of acids, practically only octadecenoic acid ($C_{18:1}$) was present.

Esters 3 were found only in extract B, and they gave, as the only group of esters, GLC peaks already when in native form, *i.e.* before transesterification. On the basis

of retention data and mainly on the basis of mass spectra individual peaks were identified as fatty acids ethyl esters. However, it cannot be excluded that these ethyl esters are artifacts formed in the course of the extraction with the ethanol–chloroform mixture.

Esters 4 were found in extract A only. After transesterification an alcohol with RRT 3·6 strongly predominated, while among acids octadecenoic acid ($C_{18:1}$) was the main component.

Esters 5 were found in extract B only. Similarly as esters *4* they may be classified among medium polar esters. After reesterification only acids could be detected by gas chromatography (see Table III).

Esters 6, equally as *esters 7*, can be considered very polar on the basis of their R_F values. They were separated only from extract B. In esters 6 the alcoholic component with RRT 3·6 predominated, in esters 7 only acids were detected (see Table III).

Secondary alcohol. Using preparative TLC on silica gel a compound with R_F 0·55 (in light petroleum–diethyl ether 9 : 1) was isolated from extract B, which gave a single peak on GLC. On the basis of its IR spectrum (3 400 cm^{-1}, —OH), mass spectrum and the spectrum of its silyl derivative it follows that it is a branched secondary alcohol ($C_{40}H_{82}O$, m.w. 578). The position and the type of branching could not be determined.

TABLE III

Groups of esters isolated from extracts A and B

Esters	R_F[e]	IR			Acids present in largest amounts[f]
		—COOR	—C=C—	$(CH_2)_n$	
1[a]	0·95	1 734	—	722	Traces $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$
2	0·81	1 734, 1 190, 1 177	3 010, 1 671, 1 650	—	$C_{18:1}$
3[b]	0·70	1 739, 1 181	3 010, 1 650	—	$C_{18:1}$, $C_{16:0}$, $C_{18:2}$, $C_{18:0}$
4[a,c]	0·55	1 745, 1 162	—	720	$C_{18:1}$, $C_{16:0}$
5[a,b]	0·50	1 745	3 020	728	$C_{18:1}$, $C_{16:0}$, $C_{18:2}$, ($C_{18:0}$)
6[a,d]	0·23	1 742	—	—	$C_{18:1}$, $C_{16:0}$, $C_{18:0}$
7[a,d]	0·05	1 742	3 010	722	$C_{18:1}$, $C_{16:0}$, $C_{18:2}$, $C_{18:0}$

[a] IR spectra were measured in KBr micro pellets; [b] isolated from extract B only; [c] found in extract A only; [d] esters with a distinct odour; [e] elution system: light petroleum–diethyl ether 9 : 1, TLC on silica gel; [f] determined by GLC after reesterification.

Triacyl glycerols together with free acids constituted the main fraction in both extracts. Their R_F value on thin layer of silica gel (0·50, light petroleum–diethyl ether 9 : 1) is identical with the R_F value of tristearyl glycerol. The IR spectrum displayed bands for the ester group (1 745 and 1 159 cm^{-1}) and for a *cis*- double bond (3 000 and 1 650 cm^{-1}). The presence of glycerol was demonstrated by gas chromatography of a sample after reesterification and subsequent silylation, by comparison with trimethylsilyl derivative of glycerol. The occurrence of individual acids (chromatographed as methyl esters) is shown in Table IV. The position of the double bonds in some of the most abundant acids of the triacyl glycerols of extract B was determined by GC-MS after hydroxylation with osmium tetroxide and subsequent silylation[9–13]. In the case of hexadecenoic acid ($C_{16:1}$) and octadecenoic acid ($C_{18:1}$) the double bond is in position 9, in eicosenic acid ($C_{20:1}$) it is in the position 11 and in octadecadienoic acid ($C_{18:2}$) the double bonds are in position 9 and 12. It may be assumed that identical acids will also be present in triacyl glycerols of extract A.

Fatty acids. The second most abundant group of substances are free acids. Their R_F value in TLC on silica gel (0·70, light petroleum–diethyl ether 7 : 3) is identical with the R_F value of stearic acid. The IR spectrum contained bands for carboxyl group (3 400–2 400, 1 742 and 1 709 cm^{-1}) and for a *cis*-double bond (3 000 cm^{-1}). The representation of individual acids, chromatographed in the form of methyl esters, is given in Table IV. The positions of the double bonds determined by GC-MS in the above described manner are identical with the positions of the double bonds of corresponding acids from triacyl glycerols. In extract B eicosatrienoic acid ($C_{20:3}$) and eicosatetraenoic acid ($C_{20:4}$) are also present in considerable amounts.

The relatively high content of free fatty acids, together with the content of triacyl glycerols evidently take part in the perfect hydrophobisation of the body surface of the animals.

Sterols are present in considerable amounts in both extracts. Their R_F value in TLC on silica gel (0·30, light petroleum–diethyl ether 7 : 3) is identical with the R_F of cholesterol. The IR spectrum contained a band for the hydroxyl group (3 620 and 1 054 cm^{-1}). In GLC of a native sample and an acetylated sample on the phases OV-101 and OV-17 two peaks were observed (80 and 15% in extract A and 63 and 37% in extract B) the retention times of which[27] were identical with those of cholesterol and desmosterol. Mass spectrometry confirmed this finding.

Heterocyclic nitrogen-containing compounds. From the last chromatographic fractions of both extracts A and B two polar compounds were eluted. After purification on a silica gel thin layer (R_F = 0·50 and 0·30, light petroleum–diethyl ether 1 : 1) each compound gave an individual peak in GLC (see Table II) and mass spectrometry showed that their composition was $C_{11}H_{13}N_3O$ and $C_9H_9N_3O_2$. A detailed proof of the structures of these compounds is the subject of the subsequent communication[28].

TABLE IV

Representation of fatty acids in extracts A and B (%)

Number of C atoms	Acids from triacyl glycerols		Free acids	
	A	B	A	B
11 : 0	—	—	0·4	—
12 : 0	—	0·2	—	—
14 : 0	0·8	0·5	—	0·1
15 : 0	0·9	0·6	0·8	0·4
16 : 0	22·7	14·9	25·5	14·0
16 : 1	7·1	4·4	2·1	1·2
17 : 0	0·5	0·5	1·4	0·7
18 : 0	4·7	3·8	18·1	9·9
18 : 1	51·1	37·1	45·6	25·2
18 : 2	4·2	21·1	1·9	17·0
19 : 0	+[a]	0·1	0·3	0·1
20 : 0	1·1	0·7	0·2	0·3
20 : 1	1·0	5·7	2·5	4·5
20 : 2	—	—	—	0·6
20 : 3	—	3·4	—	19·3
20 : 4	—	—	—	4·8
21 : 0	0·2	—	—	—
21 : 1	+	—	—	—
22 : 0	0·8	0·3	0·2	0·2
22 : 1	0·1	—	—	—
23 : 0	0·2	—	+	—
23 : 1	0·1	—	—	—
24 : 0	0·7	1·8	+	—
24 : 1	0·1	—	—	—
25	+	—	—	—
26	0·5	—	—	—
28	0·5	—	—	—
30	0·5	—	—	—
32	0·4	—	—	—
unknown	1·8	4·9	1·0	1·7
Total	100	100	100	100

[a] + means traces.

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