

FATTY ACID COMPOSITION OF THE INFUSORIAN *Paramecium caudatum*
AND *Colpoda steini* (CILIOPHORA TYPE)

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The lipids of free-living infusoria of the Ciliophora type have been little studied. The composition of the fatty acids of infusoria belonging to the genera *Tetrahymena* [1, 2] and *Paramecium* [3, 4] have been studied most widely. Recently, there has been a considerable rise of interest in the lipids of infusoria especially of free-living species. This is due to the fact that they contain a considerable amount of phosphonic and alkyl-acyl glycerophospholipids [5, 6]. Alkyl-containing phospho- and phosphonolipids include up to 83% of eicosatetraenoic acid [3]. At the present time, the genus *Paramecium* includes more than 10 species, but the lipid and fatty acid compositions of only two of them have been studied: *P. aurelia* and *P. tetraurelia* [3, 6].

Biomass from the Kuibyshev reservoir (region of the Institute of Ecology of the Volga Basin of the USSR Academy of Sciences) was obtained by the method of accumulation under standard conditions. The extraction of the lipids, the isolation of the fatty acids, and the preparation of their methyl esters were carried out as we have described previously [7, 8]. Their fatty acid compositions are given below (wt.-%, GLC):

| Acid | <i>Paramecium caudatum</i> | <i>Colpoda steini</i> |
|-----------------|----------------------------|-----------------------|
| 12:0 | 1.3 | — |
| 14:0 | 1.5 | 0.6 |
| 14:1 | 1.0 | — |
| 16:0 | 22.9 | 8.0 |
| 16:1 | 4.1 | 1.9 |
| 18:0 | 7.5 | 2.1 |
| 18:1 | 16.5 | 1.6 |
| 18:2 | 20.3 | 0.4 |
| 20:1 | 0.3 | — |
| 20:2 ω 6 | 1.3 | 4.8 |
| 20:4 ω 6 | 23.3 | 80.6 |
| Saturated | 33.2 | 10.7 |
| Monoenoic | 21.9 | 3.5 |
| Dienoic | 21.6 | 5.2 |
| Polyenoic | 23.3 | 80.6 |

The main fatty acids of *P. caudatum* are the 20:4 ω 6, 18:2, 16:0, and 18:1 types, while for the infusorium *Colpoda steini* the main acid is arachidonic, which makes up 80.6% of the total fatty acids.

Thus, two species of free-living infusoria, *Colpoda steini* and *Paramecium caudatum* have been isolated for the first time and the fatty acid compositions of both lipid extracts have been studied. It has been shown that these species contain a considerable amount of the eicosatetraenoic acid 20:4 ω 6 and can be used for its preparative isolation, while infusoria may also serve as excellent objects for investigating the metabolism of polyunsaturated acids.

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11,12-DEHYDROURSOLIC ACID LACTONE FROM LEAVES OF *Eucalyptus viminalis*

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We have continued a study of the triterpenoids of the leaves of the ribbon eucalyptus [1]. In addition to ursolic and maslinic acids, according to TLC the chloroform extract contained a less polar triterpenoid with R_f 0.8 (Silufol; chloroform-methanol (9:1)). Liquid-phase extraction of the chloroform extract with 2% sodium hydroxide solution gave the combined acid components, which were partially freed from phenolic components by treatment of a 2% chloroform solution with a 2% solution of sodium bicarbonate (5:3).

The residue was chromatographed successively on OU-A alkaline carbon (1:4), silica gel 40/100 (1:100), alumina (activity grade II, 1:100), and silica gel 40/100 (1:50). The eluents were ethanol, chloroform, chloroform-methanol (9:1), and chloroform, respectively. The presence of a desired substance in the fractions was monitored by TLC in comparison with the extract. Crystallization from petroleum ether gave the compound $C_{30}H_{46}O_3$, mp 270-272°C.

The nature of the coloration on a Silufol plate on visualization with an ethanolic solution of tungstophosphoric acid was typical for 11,12-dehydro derivatives of oleanolic and ursolic acids: a bright orange coloration changing to crimson, and then to greyish green [2]. The IR spectrum contained the absorption band of a γ -lactone (1745 cm^{-1}). The PMR spectrum showed a doublet of doublets at 3.21 ppm, $J_1 = 10.1\text{ Hz}$, $J_2 = 5\text{ Hz}$ (H-3). In the weak-field region there were doublets of doublets of a $-\text{CH}=\text{CH}-$ group at 5.95 ppm, $J_1 = 10.25\text{ Hz}$, $J_2 = 1.95\text{ Hz}$, and at 5.52 ppm, $J_1 = 10.25\text{ Hz}$, $J_2 = 3.17\text{ Hz}$.

The weaker-field signal was due to a proton experiencing additional descreening through the closeness of an oxygen function. Its splitting with a SSC of 1.95 Hz is characteristic for allyl interaction [3]. Thus, the parameters of the signals mentioned were characteristic for olefinic protons at C-11 and C-12 where a gamma-lactone was present in the 28-13 position. Doublets of two methyl groups of the seven signals of CH_3 groups showed that the compound was an ursolic acid derivative.

The mass spectrum also agreed with the structure of 11,12-dehydroursolic acid lactone. Thus, a peak with m/z 410 (11.22%) corresponded to the ion formed as the result of the elimination of CO_2 from the molecular ion, and peaks with m/z 241 (2.52%) and 169 (14.82%) to the ions formed by the successive cleavage of the C9-C10 and C7-C8 bonds and the migration of a proton (fragments from rings D, E, and A, B, respectively).

The spectral features mentioned, and also the melting point of the acetyl derivative (262°C, decomp.) correspond to the 11,12-dehydroursolic acid lactone isolated previously from the leaves of some eucalypt species other than the ribbon eucalyptus in the form of the acetate [2]. In the PMR spectrum of 11,12-dehydroursolic acid lactone, a doublet of one of the secondary CH_3 groups at 0.97 ppm had components of equal intensity, while the ratio of the intensities of the weak-field and the strong-field components of the doublet of another secondary CH_3 group at 0.92 ppm was 1:3. On this basis, it may be concluded that the signal of the methine proton interacting with this CH_3 group was located to the right of its signal, in the stronger field at $\sim 0.85\text{-}0.9\text{ ppm}$, i.e., the methine proton experienced additional screening. This condition would correspond to a proton at C-19 present below the plane of the D ring because of the rigid fixation of ring E due to the γ -lactone grouping. This proton is screened by four simple carbon-carbon bonds: C13-C18, C17-C18, C20-C21, and C20-C30. The signal of H-5 was located in an even stronger field (dd at 0.74 ppm, $J_1 = 11\text{ Hz}$, $J_2 = 3\text{ Hz}$), being screened by seven simple bonds (C3-C4, C6-C7, C1-C10, C10-C9, C4-C23, C4-C24, and C10-C25). The assignment of the H-5 signal agrees with the literature [4]. The spectrum of the substance was obtained on a Bruker WM-500 instrument.

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