

É. A. Shaftan, G. Z. Shishkov,  
N. V. Popova, V. M. Banashek,  
and A. V. Pekhov

UDC 663.958:547.915

The lipid composition of a CO<sub>2</sub> extract from industrial wastes of tea manufacture has been studied for the first time. It contains phospholipids, fatty acid monoglycerides, carotenoids, sterols, tocopherols, free acids, hydrocarbons, and waxes. The carotenoids are represented by  $\beta$ -carotene. The fatty acid compositions of the free and bound acid fractions were determined by GLC. It was found that palmitic acid predominated in the free state and stearic acid in the bound state.

Processing-wastes in the tea industry consist of coarse stems and veins of the tea leaf and also of the tea dust that settles in the cyclones during the fermentation and drying of the tea. So far these wastes have found practically no use.

We have obtained an original product — a CO<sub>2</sub> extract from a mixture of tea industry wastes — for the first time. Since liquid carbon dioxide is a weakly polar extractant, the basis of the CO<sub>2</sub> extract obtained consisted of substances with a lipoid nature and substances accompanying these. The CO<sub>2</sub> extract of the tea wastes consisted of a viscous yellow-brown grease-like mass with an odor characteristic of green tea,  $n_D^{20}$  1.4970, acid No. 26 mg KOH; completely soluble in ethanol on heating.

The group analysis of the lipoids of the CO<sub>2</sub> extract of tea wastes was carried out by the TLC method on activated Silufol plates by a known method [1]. Sunflowerseed oil was used as marker. The following were identified from literature information and the results of the comparison with the sample of sunflowerseed oil on the basis of  $R_f$  values: phospholipids ( $R_f$  0.00), monoglycerides ( $R_f$  0.06); carotenoids ( $R_f$  0.20), free sterols ( $R_f$  0.34), free fatty acids ( $R_f$  0.62), and hydrocarbons and waxes ( $R_f$  1.00). In addition, two unidentified groups of substances with  $R_f$  0.04 and 0.13 appeared.

The carotenoids in the CO<sub>2</sub> extract were investigated directly in the initial product and also in the unsaponifiable fraction of the CO<sub>2</sub> extract, likewise by a known method [2] using PC in solvent system 2 and TLC in system 3. A single spot appeared on each chromatogram with an  $R_f$  value close to unity. Spectrophotometry of the eluate in the near-UV and visible regions of the spectrum showed that the product contained  $\beta$ -carotene in the cis form. The amount of the latter in the CO<sub>2</sub> extract was 0.023%.

The sum of the tocopherols in the unsaponifiable fraction of the CO<sub>2</sub> extract was determined by Devyatinn's method [3] with bi- $\alpha$ -pyridyl and ferric chloride; it amounted to 0.100%. The sum of the sterols from the unsaponifiable fraction determined by precipitation with digitonin [4] also amounted to 0.100%.

The fatty acid compositions of the fractions of free and bound acids (the acid residues of the esters) were investigated by the GLC of the corresponding methyl esters. The results of the analysis are given in Table 1, from which it can be seen that the compositions of the acids in the free and bound states differed both qualitative and quantitatively, 19 acids being found in the free state and 30 in the bound state. The main component of the fraction of free acid was palmitic acid and an unimportant position was occupied by the unsaturated acids oleic and linoleic (in combination, about 34%). Among the bound acids stearic predominated, and unsaturated acids were present in very small amounts. It is characteristic that, according to the TLC of the lipoids, the bound acids were present in the CO<sub>2</sub> extract only in the form of monoglycerides. It must also be mentioned that the fraction of bound acids, unlike the free-acid fraction, contained a considerable amount of iso acids.

---

Scientific-Research Institute of the Food Industry, Krasnodar. Translated from *Khimiya Prirodnikh Soedinenii*, No. 2, pp. 181-184, March-April, 1985. Original article submitted May 7, 1984.

TABLE 1. Composition of the Acids of a CO<sub>2</sub> Extract of Tea Wastes, % on the Total

Acid	Free acids	Acid residues after the hydrolysis of the esters
X <sub>1</sub>	—	2,0
X <sub>2</sub>	2,0	1,4
X <sub>3</sub>	2,1	3,4
X <sub>4</sub>	Tr.	1,0
X <sub>5</sub>	—	1,0
Butanoic 4:0	Tr.	2,6
Hexanoic 6:0	Tr.	Tr.
Octanoic 8:0	—	Tr.
Nonanoic 9:0	Tr.	Tr.
Decanoic 10:0	Tr.	2,0
X <sub>6</sub>	—	0,1
Tetradecanoic 14:0	—	Tr.
X <sub>7</sub>	—	3,3
Pentadecanoic 15:0	—	Tr.
β-Methylpentadecanoic, iso-16:0	—	0,3
X <sub>8</sub>	Tr.	Tr.
X <sub>9</sub>	Tr.	0,2
X <sub>10</sub>	—	0,1
X <sub>11</sub>	—	Tr.
X <sub>12</sub>	—	Tr.
Hexadecanoic 16:0	54,1	15,4
Palmitoleic 16:1	Tr.	0,1
1,4-Methylhexadecanoic, iso-17:0	0,2	14,2
Methylheptadecanoic, iso-18:0	Tr.	5,9
Octadecanoic	6,8	45,7
Oleic	15,4	0,4
Linoleic 18:2	18,5	0,4
Linolenic 18:3	Tr.	Tr.
X <sub>13</sub>	—	0,3
X <sub>14</sub>	0,5	0,2
Σ <sub>sat</sub>	66,1	99,1
Σ <sub>unsat</sub>	33,9	0,9
Σ iso acids	0,2	20,4

Note. X<sub>1</sub>, X<sub>2</sub>, ..., X<sub>n</sub> unidentified acids.

The presence of caffeine — an alkaloid coextracted with the lipoids of the tea plant — was demonstrated in the CO<sub>2</sub> extract of the tea production wastes spectrophotometrically from the nature of the absorption in the UV region at 275 nm.

#### EXPERIMENTAL

General Observations. For thin-layer chromatography we used Silufol plates activated in a thermostat at 120°C for 30 min. Paper chromatography was carried out on Leningradskaya bystraya ["Leningrad rapid"] chromatographic paper. The following solvent systems were used for chromatography: 1) petroleum ether—diethyl ether—glacial acetic acid (85:15:1); 2) hexane; 3) hexane—diethyl ether (3:7). The revealing agent for system 1 was a 5% ethanolic solution of molybdophosphoric acid followed by heating at 100°C for 5 min.

Spectrophotometry was performed on a Specord M40 instrument, and photolorimetry on an FEK-56 instrument.

GLC was performed on a Chrom-5 chromatograph with a flame-ionization detector. Conditions of separation: spiral stainless-steel column 3000 × 3 mm; stationary phase Celite 545 with 20% PEGS; carrier gas argon at the rate of 80 ml/min. The temperature of the column was 195°C, that of the evaporator 250°C, and that of the detector 250°C.

Isolation of the Free and Bound Acids and of the Unsaponifiable Fraction. A sample of the CO<sub>2</sub> extract was dissolved in a mixture of ethanol and ether, and the free acids were titrated with 0.1 N NaOH in the presence of phenolphthalein until a permanent color was obtained. The neutral fraction was extracted from the reaction mixture with diethyl ether (4–5 × 30 ml). The ethereal extracts were combined, washed with water to neutrality, dried over calcined Na<sub>2</sub>SO<sub>4</sub>, and evaporated to give a neutral extract.

The soaps (the ethanol–ether–water layer) and the wash-waters were combined, evaporated on the water bath until the smell of ethanol had disappeared, and acidified with 10% HCl to

a distinctly acid reaction. The liberated acids were extracted with diethyl ether, and the extracts were dried over calcined  $\text{Na}_2\text{SO}_4$  and evaporated. This gave the free fatty acid fraction.

The neutral extract was subjected to alkaline hydrolysis with 10% ethanolic NaOH on the water bath at  $80^\circ\text{C}$  for 60 min.

The unsaponifiable fraction was extracted from the cooled hydrolysate repeatedly with diethyl ether. The ethereal extracts were combined, washed with water to neutrality, dried over calcined  $\text{Na}_2\text{SO}_4$ , and evaporated to give the unsaponifiable fraction.

The soaps were combined with the wash-waters from the unsaponifiables and the mixture was acidified with 10% HCl to a distinctly acid reaction. The acid components of the esters (the bound acids) were extracted with diethyl ether. The ethereal extracts were combined, dried over calcined  $\text{Na}_2\text{SO}_4$ , and evaporated to give the bound-acid fraction.

The free and bound acids were methylated separately and the methyl esters were analyzed by GLC.

#### SUMMARY

1. The lipid components of a new product — a  $\text{CO}_2$  extract from tea production wastes — have been studied.

2. The group composition of the lipoids has been established and the qualitative and quantitative characteristics have been given for individual groups of lipoids: carotenoids, tocopherols, sterols, and fatty acids, and also the alkaloid caffeine accompanying them.

#### LITERATURE CITED

1. M. S. Umanskii, in: Abstracts of Lectures at the IVth Scientific Conference of Young Scientific Biologists [in Russian], Moscow (1960), p. 65.
2. B. G. Savinov, Carotene [in Russian], Kiev (1948).
3. V. A. Devyatnin, Methods of Chemical Analysis in the Manufacture of Vitamins [in Russian], Moscow (1964).
4. Handbook on Methods of Investigation, Technical and Chemical Control, and the Accounting of Production in the Oils and Fats Industry [in Russian], Leningrad, Part 1, Book 2 (1967), p. 320.

#### STEREOCHEMISTRY OF TERPENOID COUMARINS.

#### CRYSTAL AND MOLECULAR STRUCTURE OF SAMARCANDIN

S. M. Nasirov, A. I. Saidkhodzhaev,  
T. Kh. Khasanov, M. R. Yagudaev, and  
V. M. Malikov

UDC 547.582

The absolute configuration of samarcandin has been established on the basis of the results of a complete X-ray investigation. The absolute configurations of conferol, moscharol, badrakemin, and coladonin, and the absolute configurations of feshurin, nevskin, and isosamarcandin have been refined.

At the present time, more than 40 terpenoid coumarins with a bicycloprenyl residue have been isolated from plants of the genera *Ferula* and *Colladonia*, and these have been subdivided into two groups according to the orientation of the aryloxymethylene group at  $\text{C}_9$ : with the substituent  $\text{C}_9\text{—CH}_2\text{OAr}$  orientated axially (group I), and equatorially (group II). The absolute configurations of representatives of coumarins of group I — farnesiferol A and gummosin — have been determined on the basis of chemical transformations and the methods of optical rota-

---

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek, SSR, Tashkent. Translated from *Khimiya Prirodnikh Soedinenii*, No. 2, pp. 184-190, March-April, 1985. Original article submitted December 19, 1983.