Fatty Acid Profiles of Three Marine Species: *Thorsonia investigatoris*, *Chloeia parva* and *Sepia aculeata*

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IN continuation of our search[1] for marine bioactive substances we have investigated the fatty acid composition of the lipids of three marine species: *Thorsonia investigatoris*[2], *Chloeia parva*[2] and *Sepia aculeata*[3].

The lipids of the above species were extracted according to the reported method[4]. The fatty acids obtained after saponification of the total lipid were methylated and the methyl esters were analysed by glc. The results (Table 1) reveal that besides the typical fatty acids, the *n*3 and *n*6 groups of polyunsaturated fatty acids are present in significant amounts. The interesting feature is that the fatty acids 20 : 5*n*3 and 22 : 6*n*3 are present in significantly high level in *S. aculeata* in comparison to other two species.

TABLE 1—COMPOSITION (WT. %) OF FATTY ACIDS OF THE TOTAL LIPIDS OF THREE SPECIMENS*

Fatty acid	Percentage composition		
	T i.	*C p.*	*S.a.*
12 : 0	0.54	0.14	–
14 : 0,iso	–	0.04	–
14 : 0	4 62	3 64	4.7
14 : 1[a]	5.16	0.42	–
15 : 0	3.75	1.39	–
16 : 0,iso	2.14	0.54	–
16 : 0	12.27	16.90	32.9
16 : 1*n*7	9.06	7.48	–
17 : 0	2.14	3.01	–
18 : 0,iso	1.13	3.50	–
18 : 0	9.76	7.18	10 4
18 : 1*n*9	4 96	10.45	6 6
18 : 2*n*6	2.03	6.09	1.2
20 : 0	0.39	–	–
18 : 3*n*6	–	2.08	–
18 : 3*n*3	2.47	11.32	–
	3.40[b]	0.85[b]	–
	–	2.50[b]	–
20 : 3*n*6	2.65	1.79	–
20 : 3*n*3	0.61	–	–
20 : 4*n*6	3.28	6.81	4 0
20 : 4*n*3	0.43	–	–
22 : 1[a]	–	2.53	–
20 : 5*n*3	1.06	6.08	14.7
22 : 3*n*6	0.44	–	–
22 : 4*n*6	17.12	0.55	–
22 : 5*n*6	3.27	0.82	1.0
22 : 5*n*3	3.41	1.69	1.2
22 : 6*n*3	3.86	2.20	23.3

* *T. i.* ≡ *T. investigatoris*; *C. p.* ≡ *C. parva*; *S. a* ≡ *S. aculeata*.

[a]Position of the double bond not known.

[b]Could not be identified.

Experimental

The animals were collected from the coastal region of the Bay of Bengal at Digha in September 1986. Voucher specimens have been deposited in the Zoological Survey of India, Calcutta.

Extraction of lipids and preparation of fatty acid methyl esters (FAME) : The sliced animal was blended with $CHCl_3-MeOH$ (1 : 2, v/v) in a homogeniser for 10 min. The homogenate was filtered and the residue was further homogenised for 2 min with $CHCl_3-MeOH-H_2O$ (1 : 2 : 0.8, v/v). It was then filtered and the residue was again extracted as in the first step. The combined filtrates were diluted with $CHCl_3-H_2O$ (1 : 1, v/v) and the lower organic phase was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated in a rotary evaporator at 30°. The total lipid so obtained was hydrolysed by refluxing with 4% methanolic KOH for 2.3 h in N_2-atmosphere. After removal of the solvent the viscous mass thus obtained was thoroughly triturated with Et_2O to give the nonsaponifiables. The residue was dissolved in H_2O and then acidified with $4N$ H_2SO_4 and the free fatty acids were extracted with Et_2O. The free acids were methylated with diazomethane.

A Hewlett & Packard gas chromatograph with dual column and dual flame ionisation detector was used; column : chromosorb (100 – 120 mesh) coated with 15% DEGS ; column temperature (isothermal) : 190° ; injection port and detection temperature 200° ; carrier gas : N_2 ; flow rate : 35 ml min^{-1}. The peaks were identified using cod liver oil fatty acid methyl esters as secondary standards[5] and from the plots of log r.r.t. vs carbon number of known fatty acid methyl esters. The composition of fatty acid methyl esters was determined by computer.

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References

1 P. K. DATTA, A K. RAY, A. K BARUA, S. K. CHOWDHURI and A. PATRA, *J. Nat. Prod.*, in the press; A. K. RAY, P. K. DATTA, T. DAS, P. BHATTACHARYYA, A. K. BARUA, A. PATRA and A. ACHARYYA, *J. Nat. Prod.*, accepted.

2. P. K. DATTA, Ph. D. Thesis, Jadavpur University, 1986.

3. A. K. RAY, Ph. D. Thesis, Calcutta University, 1987.

4 E. G. BLIGH and W. J. DYER, *Can. J. Biochem. Physiol*, 1959, **37**, 911.

5. R. G. ACKMAN and R. D. BURGER, *J. Am. Oil Chem. Soc.*, 1965, **42**, 38.

Separation of Indium as Ascorbate Complex by Extraction with Aliquat 336S

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LIQUID anion-exchangers find extensive use from mineral and organic acid media in separation chemistry[1]. The present paper reports the separation of indium as ascorbate complex by extraction with aliquat 336S from associated elements.

Experimental

Pure indium (0.5 g, Indium Corporation of America) dissolved in concentrated hydrochloric acid (5 ml) was diluted to 100 ml with distilled water. This solution was standardised complexometrically using 4-(pyridylazonaphthol). It contained 5 mg ml^{-1} of indium. A solution containing 50 μg ml^{-1} indium was prepared by appropriate dilution with water.

Indium ascorbate complex was formed by addition of 0.01 M of ascorbic acid to indium (50 μg) at pH 4.5. The complex was extracted with 0.1 M Aliquat 336S in xylene in ascorbate media by shaking for 10 min. The extracted indum was stripped from organic phase with nitric acid. The recovered indium was determined spectrophotometrically using 4-(2-pyridylazoresorcinol) as a chromogenic ligand. The absorbance of the red colored complex was measured at 510 nm against reagent blank[2]. The amount of indium was computed from calibration curve.

Results and Discussion

The pH studies of extraction of indium as a ascorbate complex showed that pH 4 – 5 is suitable for quantitative extraction with 0.1 M Aliquat 336S in xylene. Amberlite LA-1 or LA-2 and TOA could not extract indium quantitatively at any pH. The optimum concentration of Aliquat 336S found for quantitative extraction was at and above 0.1 M, whereas the concentration of ascorbic acid for complete complexation was at and above 8×10^{-3} M.

Chloroform and xylene were found suitable for quantitative extraction of indium as ascorbate complex, the percentage extraction being 98.9 and 99.9 respectively. The best stripping agent for recovery of indium from organic phase after extraction was 0.5 M nitric acid. Hydrochloric, sulphuric and hydrobromic acids, however, could not strip indium quantitatively.

The nature of composition of ion-association complex was found out from the plots of log D vs log [Aliquat 336S] at a fixed [ascorbic acid] and log D vs log [ascorbic acid] at constant [Aliquat 336S].

The slopes were 1.2 and 1.3 respectively indicating (R_4N^+ $In(OH)_3$ $Ascorb^-$) as probable species and the extracted species chlorocomplex being (R_4N^+ $InCl_4$).

It is possible to separate indium as ascorbate complex from alkali and alkaline earth, thallium, irom and lanthanum as these elements could not form negatively charged ascorbate complex.

It was possible to separate indium from the binary mixtures (in ratio of 1 : 30) containing Mn, Co, Ni, Cu, Cd, Al, Zn and Hg. When these elements were studied they were first washed with water and In was stripped with 0.5 *M* nitric acid. Zr, Ti, V^{IV}, Th, Ga, Sc and Mo from negatively charged complexes hence they were quantitatively extracted. Their separation from In was carried out in mineral acid media. When the above elements were individually extracted with indium, stripping was carried with 4 *M* hydrochloric acid. At this stage indium forms anionic chlorocomplex hence remains in organic phase, whereas other elements are left in aqueous phase.

The developed method can be applied in case of ternary separation. The best example is the separation of Al/Tl, Ga and In. The tolerance limit of Al and Tl in the mixture-set was 1000 and 1500 mg. When a mixture containing these three metals was shaken with 0.1*M* Aliquat 336S from ascorbic acid (0.01*M*), Al/Tl remained unextracted in aqueous phase. The extracted Ga and In were separated by stripping Ga first with 2.5*M* hydrobromic acid and finally stripping In with 0.5*M* nitric acid. It is possible to carry out separation of Al/Tl, Ga and In in the ratio of 3 : 1 : 1 (Table 1).

TABLE 1—SEPARATION OF INDIUM IN TERNARY MIXTURES

Sl. no.	Element	Taken μg	Found μg	Recovery %	Stripping agents
1.	Al	201.0	201.0	100	H_2O
	Ga	51.0	51.0	100	2.5 *M* HBr
	In	50.0	50.0	100	0.5 *M* HNO_3
2.	Tl^I	145.0	144.0	99.3	H_2O
	Ga	51.2	51.2	100	2.5 *M* HBr
	In	50.0	50.0	100	0.5 *M* HNO_3

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References

1. H. GREEN, *Talanta*, 1964, **11**, 561 ; 1973, **20**, 139 ; A. M. KAPLUNOVA, N. I. ERSHOVE and T. A. BOLSHOVA, *Zh. Anal. Khim.*, 1978, **33**, 1940 ; S. D. SHETE and V. M. SHINDE, *Analyst*, 1982, **107**, 225 ; R. R. RAO and S. M. KHOPKAR, *Indian J. Chem.*, *Sect. A*, 1982, **22**, 398 ; C. P. VIBHUTE and S. M. KHOPKAR, *Analyst*, 1985, **111**, 435.
2. F. D. SNELL, "Photometric and Fluorometric Methods of Analysis", Wiley, New York, 1978, Parts I and II.