Chromatographic separation of N-2,4-dinitrophenylhydrazides of lower fatty acids

Chromatographic separation of the lower fatty acids has previously been carried out by the author, using a column of Amberlite IRC 50 as adsorbent and a mixture of acetone, methyl ethyl ketone and water as eluent[1]. Since this method did not permit the separation of isobutyric acid from *n*-butyric acid and of α-methylbutyric acid from isovaleric acid, and since the low efficiency of the method seemed to be due to the weaker adsorption of fatty acids on Amberlite IRC 50, the separation of these isomers as their N-2,4-dinitrophenylhydrazides* was investigated.

Amberlite IRC 50 H-form (pulverized and screened as described earlier[1]) was washed on a glass filter with a solvent composed of methyl ethyl ketone, acetone and water (2 : 1 : 9 by vol.) and suspended in 2 vols. of the same solvent. The suspension was

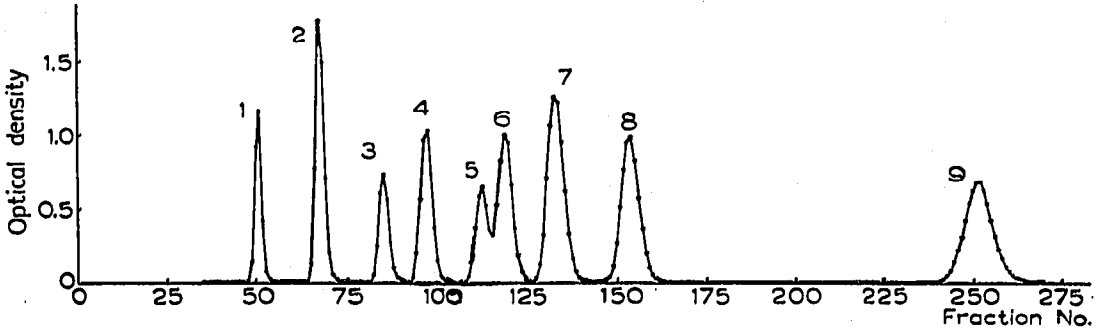

Fig. 1. Elution curve of N-2,4-dinitrophenylhydrazides of lower fatty acids. The compounds in the order of their elution from the column, are: N-2,4-dinitrophenylhydrazide of acetic acid (1), of propionic acid (2), of isobutyric acid (3), of *n*-butyric acid (4), of trimethylacetic acid (5), of α-methylbutyric acid (6), of isovaleric acid (7), of *n*-valeric acid (8) and of caproic acid (9). The N-2,4-dinitrophenylhydrazide of formic acid overlapped that of acetic acid.

poured into a chromatographic tube and allowed to settle under gravity. A column of 0.76 cm in diameter and 118 cm in height was used. The N-2,4-dinitrophenylhydrazides were dissolved in solvent of the same composition as that used for the packing of the column and 1 ml of the solution was placed on the column. After the solution had drained into the column, the inner wall of the chromatographic tube was washed with 0.1 to 0.2 ml of the solvent and the N-2,4-dinitrophenylhydrazides were eluted with the same solvent. The effluent was collected in fractions of 3.1 ml in test tubes graduated at 3.5 ml. The flow rate was 4.5 ml/h and the ambient temperature was 28–31°. Each fraction was diluted to 3.5 ml with the solvent used for the chromatographic separation. Then the ultraviolet absorption of each fraction was measured at 340 mμ using a Beckman model DU quartz spectrophotometer (Fig. 1).

* The N-2,4-dinitrophenylhydrazides were prepared: (1) by boiling 2,4-dinitrophenylhydrazine with excess fatty acid[2] (N-2,4-dinitrophenylhydrazide of formic, propionic and *n*-butyric acids); (2) by boiling 2,4-dinitrophenylhydrazine and fatty acid in 5 *N* sulfuric acid[3] (N-2,4-dinitrophenylhydrazide of trimethylacetic acid); and (3) by dinitrophenylation of fatty acid hydrazide with 2,4-dinitrofluorobenzene[4] (N-2,4-dinitrophenylhydrazide of isobutyric, isovaleric, α-methylbutyric, *n*-valeric and caproic acids). The details will be reported elsewhere.

TABLE I

RECOVERY OF N-2,4-DINITROPHENYLHYDRAZIDES OF LOWER FATTY ACIDS FROM THE CHROMATOGRAPHIC COLUMN

N-2,4-Dinitrophenylhydrazide	*Added (μg)*	*Recovered (μg)*	*Recovery (%)*
Acetic acid	160	168	105
Propionic acid	305	320	105
Isobutyric acid	176	173	98
n-Butyric acid	269	272	101
Trimethylacetic acid	204	197	97
a-Methylbutyric acid	315	330	105
Isovaleric acid	461	482	105
n-Valeric acid	390	403	103
Caproic acid	441	446	101

The elution sequence was similar to that of the free fatty acids from a column of Amberlite IRC 50, but the efficiency was increased, that is the two isomers of butyric acid and the four isomers of pentanoic acid were separated as their N-2,4-dinitrophenylhydrazides.

Department of Genetics, Osaka University Medical School, Osaka (Japan) TOKUICHIRO SEKI

[1] T. SEKI, *J. Biochem.* (*Tokyo*), 45 (1958) 855.
[2] TH. CURTIUS, *J. prakt. Chem.*, [2] 76 (1907) 370.
[3] J. CEREZO AND E. OLAY, *Anales soc. espan. fis. quim.*, 32 (1934) 1090; *C. A.*, 29 (1935) 2932[3].
[4] K. OHNO, *J. Biochem.* (*Tokyo*), 40 (1953) 621.

Received September 7th, 1959

J. Chromatog., 3 (1960) 376–377

Chromatographic detection of sugars of growing cartilage

In recent years, the intense metabolic activity of growing cartilage has been extensively investigated[1]; a qualitative and quantitative study of the composition of this tissue seems therefore highly desirable. In the present paper a report is presented of a qualitative examination of the sugar content of growing cartilage.

Investigations were carried out on 40 days old albino rats and on 50 days old rabbits. Cartilage samples were cut out from the proximal end of the "tibia", carefully washed with water and hydrolysed for 10 h in *N*/10 HCl at 100°; 1 ml of acid solution was used per 10 mg of cartilage. The hydrolysate was filtered and evaporated to dryness under vacuum at 50°. The dry residue was repeatedly dissolved in a small amount of water and evaporated to dryness until the pH of the solution was about 5. The residue was then dissolved in a small volume of distilled water and applied to the