88. On Malignolipin. VI

*Column Chromatographic Isolation of Malignolipin**

By Takekazu KÔSAKI, Shinya NAKAGAWA, and Toshiko SAKA

Department of Biochemistry,
Mie Prefectural University School of Medicine, Tsu
(Comm. by Y. KUNO, M.J.A., June 13, 1960)

As the column chromatographic method was successful in the isolation of various lipids, especially of phospholipids, it was examined whether it would be available for the isolation of malignolipin from tissues and blood which make up the weak point in the method of its detection¹⁾ and isolation²⁾ previously reported.

In the column chromatography of malignolipin, neutral solvents can only be applied, but not acid solvent mixtures, as in the case of other phospholipids, because it is very labile in even very weak acid medium, different from the other phospholipids. For the identification of malignolipin, a paper chromatography followed by a ninhydrin spray, a picrate formation, a detection of phosphorus, spermine, choline, and fatty acid was applied.

Samples are extracted with boiling ethanol, and the extract is dried in vacuo. The dried residue is extracted with absolute ethanol and the extract is mixed with 2 vols of acetone. The precipitate is dissolved in various organic solvents and is used as the starting material, because those procedures were ascertained not to be omitted.

The behavior of malignolipin against various solvents and adsorbents was found to be as shown in the table. Then, the gradient elution of malignolipin with ether, ethanol, and methanol from calcium carbonate, powdered cellulose, and silicic acid was examined. In case of silicic acid, malignolipin can not be completely separated from other substances, but it can be completely separated from impurities other than spermine in case of calcium carbonate and from spermine in case of cellulose. Thus, the following method was devised.

Ether solution of starting material is put on a column of calcium carbonate at 0°C, washed well with ether till the wash ether is ninhydrin negative, then with a mixture of ether and ethanol (7:3), and eluted with absolute ethanol. The ethanol elute is concentrated to 1/5 vol, and the concentrate is mixed with 3/7 vols of ether. The

*) In part, aided by Grant No. C-4795 from the National Institute of Health, United States Public Health Service, and also by Grants from the Ministry of Education, Shionogi Co., Waksman Foundation of Japan Inc., and Upjohn Co.

1) Kôsaiki, T., et al.: Proc. Japan Acad., **34**, 293 (1958).

2) Kôsaiki, T., et al.: Science, **127**, 1176 (1958).

Elution of malignolipin at 0°C

Adsorbents Solvents	Silicic acid	Aluminium oxide	Calcium carbonate	Cellulose	Magnesium silicate
Petroleum ether	—	—	—	—	—
Ether	—	—	—	—	—
Acetone	—	—	—	—	—
Dioxane	—	—	—	—	—
Methylal	—	—	—	—	—
Pyridine	—	—	—	—	—
Ethanol	++	—	++	+	—
Methanol	++	—	++	++	—

mixture is put on a column of cellulose at 0°C. It is washed well with a mixture of ether and ethanol (3:7) till ninhydrin negative, and malignolipin is eluted with absolute methanol. If the column chromatography is carried on at 0°C and avoiding moisture, cellulose column chromatography can be omitted. That is to say, practically pure malignolipin is eluted with absolute ethanol from the calcium carbonate column loaded with ether solution of the material and washed well with ether, then with a mixture of ether and ethanol (3:7).

With this method, malignolipin can always be obtained in pure state from Ehrlich's cancer ascites, other malignant tumors and blood of cancer patients, but no trace of malignolipin can be isolated from blood of normal persons and liver of normal guinea pigs and rabbits. Thus, the column chromatographic separation of malignolipin from other substances contained in some tissues is ascertained to be possible, indicating that malignolipin is a specific substance of properties different from those substances contained in normal tissues.

However, the method depends particularly not only on the tightness of packed adsorbent, but also and seriously on the purity of the solvents. The yield is not too good, being in main due to the decomposition of malignolipin on the column.

Thus, the column chromatographic isolation of malignolipin is only to indicate the specificity of malignolipin, but can not be applied to the practical use, both for its isolation from malignant tumors and its detection or measurement in various tissues.