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Purification of A New Bifidus Factor from Carrot Root

Since the growth prompting effect of carrot (*Daucus carota* L.) on *Lactobacillus bifidus* was discovered by Ota, *et al.*,[1] we have been co-operating with them in investigation of the bifidus factor in carrot root.

The purification of the factor has been carried out using a strain of *L. bifidus* isolated by Negishi[2,3] for bioassay. After preliminary tests, a tentative purification system was adopted as follows.

Carrot Powder (1)[a)]
↓ extd. with MeOH
MeOH extract (1)
↓ precipitated with $Ca(OAc)_2$
Precipitate (5)
↓ adjusted pH to 3, adsorbed on charcoal column, eluted with MeOH
Effluent (30)
↓ extd. with acetone
Residue (40)
↓ chromatographed on DEAE cellulose stepwisely with $(NH_4)_2CO_3$ soln.
Active fraction (250)
↓ rechromatographed gradiently on DEAE cellulose
Active fraction (600)
↓ chromatographed on DEAE Sephadex gradiently with $(NH_4)_2CO_3$ soln.
Active fraction (1,500)
↓ rechromatographed on DEAE Sephadex
Active fraction (9,000)
↓ rechromatographed on DEAE Sephadex
Active fraction (40,000)

a) Specific activity

By this method, 1.7 mg. of substance which possessed a 40,000 times specific activity as much as that of the original material was obtained from 100 kg. of carrot powder. This substance, still impure, gave sufficient growth of the bifidus strain even at a concentration of 0.05 μg./ml. The factor, dialysable with the cellophane membrance, very soluble in water and scarcely or not soluble in organic solvents, behaved as an acid even in a medium of pH 1.5.

These characteristics demonstrate that the factor obtained from carrot root is a new factor which differs from those previously reported.[4~6]

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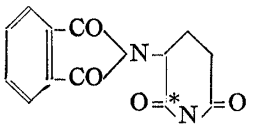
Metabolic Fate of Thalidomide in Rats*1

The metabolic fate of thalidomide is of great interest from the pharmacological point of view, because its teratogenic action could be due to any of the metabolites. Several papers have appeared recently on its metabolism.[1~5] This paper reports the biological oxidation of thalidomide labelled with ^{14}C in one of the carbonyl groups of the glutarimide moiety and the excretion pattern of the drug with ^{14}C in the phthalloyl moiety.

The ^{14}C-thalidomide was prepared by the modified Beckmann's method. The positions of the radioactive carbon (*) are illustrated by the following formulae.



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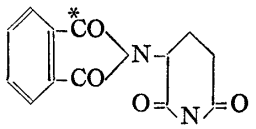

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The chemical and radiochemical purity was determined by radiopaperchromatography*2 and the melting point, elemental analysis, and infrared and ultraviolet spectra also supported the purity.

The results of measurement of expiratory radioactive carbon dioxide in rats following a single oral dose are shown in Table I.

Table I.

	Rat weight (g.) male	Experimental period (hr.)	Radioactivity of $^{14}CO_2$ obtained (d.p.m.)	Rate of decarboxylation %
Ex. 1	300	11	7.4×10^3	0.05
Ex. 2	300	11	2.7×10^4	0.08

*1 This paper was partly reported at the 19th Annual Meeting of Pharmaceutical Society of Japan (Tokyo, April 5, 1964).

*2 Solvent: (1) *n*-AmOH–Pyridine–H_2O, 7:7:6. (2) DMF–MeOH–H_2O, 20:70:5.

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