room temperature. Addition of water afforded a crude product, which, after drying and recrystallization 4 times from acetone–petroleum ether (30–70° fraction), yielded 13 mg. of pure V, m.p. 195–196°, $[\alpha]^{25}D$ +81.5° (acetone), λ_{max}^{MeOH} 239 mμ (ε 17,000); λ_{max}^{KBr} 2.84, 5.74, 5.98, 6.12, 6.18, and a plateau at 8.00–8.12 μ.

Anal. Calcd. for $C_{25}H_{31}FO_7$: C, 64.92; H, 6.76. Found: C, 65.14; H, 7.02.

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Peptinogan, a Polypeptide Moiety of Actinogan with Antitumor Properties

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Recently, we reported some of the biological and physicochemical properties of actinogan,[1] a glycoprotein derived from an actinomyces culture. During an investigation of the structure of actinogan we separated the polypeptide and the carbohydrate moieties by treatment with trichloroacetic acid.[2] The polypeptide has been named peptinogan.

The various biological properties of actinogan, such as pyrogenicity, inhibition of rodent tumors, protection against bacterial infections, enhancement of horse serum sensitization in mice, and tissue culture inhibition were found to be associated with peptinogan. This polypeptide may be preferable to the carbohydrate-containing compound as a potential antitumor agent because of improved stability and solubility and a more favorable ratio of toxic to effective dose. The carbohydrate fraction was inactive against Sarcoma 180 as well as against Staphylococcus infections in mice.

Experimental

Preparation and Purification.—A 2% aqueous solution of actinogan was treated with an equal volume of 50% trichloroacetic acid. The precipitate was suspended in water and dialyzed. The nondialyzable supension was then centrifuged, and the supernatant liquid containing the peptinogan was freeze-dried. The yield was about 10% based on the weight of actinogan; the product did not give a Molisch test and, on acid hydrolysis and chromatography of the hydrolyzate on thin-layer plates,[3] it appeared to contain at least 10 amino acids. The supernatant liquid from the trichloroacetic acid treatment, on dilution with an equal volume of ethanol, yielded a nitrogen-free carbohydrate, 57% of the weight of actinogan.

Peptinogan was further purified by chromatography on sulfoethyl cellulose[4] in the hydrogen form. A solution of 400 mg. of the polypeptide in 100 ml. of water was placed on a column of 8 g. of the exchanger; after washing with water the active material was eluted with a pH 8 buffer solution, 0.1 *M* with respect to sodium chloride and 0.05 *M* to sodium phosphate. Dialysis and freeze-drying of the fractions showing absorption at 270 mμ afforded 150 mg. of the polypeptide which inhibited S180 in mice at 2 μg. per mouse per day.

Finally this material was subjected to continuous electrophoresis, employing 600 v. in a Beckman CP apparatus and a pH 6.5 buffer consisting of 5% pyridine and 0.2% acetic acid in water. The peptide under these conditions behaved as a cation. The most active material gave a positive response against S 180 and against Carcinoma 755 in mice at 0.2 μg./day per animal.

Chemical and Physical Properties.—Peptinogan has a molecular weight of 15,000 ±20%, as determined from ultracentrifuge data. It is excluded on Sephadex G75[5] and retarded on G100.

Anal. Found: C, 50.0; H, 6.82; N, 14.8, 15.1.

It decomposes at about 240° without melting. It is soluble in water to the extent of 35 mg./ml. and insoluble in common organic solvents including glacial acetic acid, dimethylformamide, and dioxane. It is precipitated from aqueous solution with 60% ethanol or 0.3 *M* ammonium sulfate. On electrophoresis in phosphate buffer up to pH 8.2 and in sodium tetraborate–sodium hydroxide buffer from pH 8.4 to 9.0 the polypeptide behaved as a cation. A single band was obtained. At higher pH values it moved by endoosmosis in the same position as neutral yellow (Apolon®).[6] The polypeptide was detected by spraying with hypochlorite and starch–iodide.[7]

The ultraviolet spectrum in water shows one peak at 270 mμ with an absorptivity of 1.68. The infrared spectrum has bands at 2.8, 3.2, 3.4, 5.9, 6.2, 6.3, and 6.7 μ.; $[\alpha]D$ −53.7° (*c* 1, water).

The peptinogan with the greatest antitumor activity contained no Molisch-positive material. The Pauly, Sakaguchi, and diacetyl tests were positive. It was resistant to trypsin, peptidase, and carboxypeptidase but was made reactive to trypsin and peptidase by prior treatment with urea.

The percentages of amino acids were found by an analytical ion-exchange technique[8] and are listed in Table I.

TABLE I

	g./100 g.		g./100 g.		g./100 g.
Aspartic acid	11.38	1/2 Cystine	1.74	Lysine	13.23
Threonine	8.12	Valine	8.03	Histidine	0.64
Serine	5.01	Methionine	1.29	Arginine	6.53
Glutamic acid	10.17	Isoleucine	5.66		
Proline	6.50	Leucine	7.89		
Glycine	7.12	Tyrosine	3.32		
Alanine	7.85	Phenylalanine	3.04		

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(1) H. Schmitz, W. T. Bradner, A. Gourevitch, B. Heinemann, K. E. Price, J. Lein, and I. R. Hooper, *Cancer Res.*, **22**, 163 (1962).

(2) M. Ikawa, J. B. Koepfli, G. Mudd, and C. Nieman, *J. Natl. Cancer Inst.*, **13**, 157 (1952).

(3) M. Brenner, *Experientia*, **16**, 337 (1960).

(4) Bio-Rad Laboratories, Richmond, California.

(5) Pharmacia Corp., Upsala, Sweden.

(6) Obtained from Microchemical Specialties Co., Berkeley, California.

(7) R. H. Mazur, B. W. Ellis, and P. S. Cammarata, *J. Biol. Chem.*, **237**, 619 (1961).

(8) Determined by Analytica Corporation, New York 16, New York.