

G-10 gel appears to be the same in aqueous methanol and in water, in contrast to Sephadex G-25 and the polyacrylamide gel Bio-Gel P 2, which both shrink in the presence of methanol. These gels are also weaker adsorbers than Sephadex G-10 and no useful separation was observed when they were tested with eluents of constant methanol content (50%). The above method has been found especially useful for the separation of simpler phenol derivatives from flavonoid glycosides, but quite often separation of flavonoid mono- and diglycosides is also possible.

*Experimental.* Columns (1 × 50 cm) were prepared either in dilute aqueous sodium chloride solution in which case the column was washed overnight with the eluent, or directly in the elution medium. 0.1–0.2 mg of each glycoside or a mixture containing the same amount of each compound in 1 ml of eluent was applied to the column. Elution was carried out by gravity feed at a flow rate of 5–10 ml/min. 5–6 ml fractions were collected and analysed both spectrophotometrically and chromatographically (PC, solvent: butanol-acetic acid-water, 6:1:2, TLC on silica gel, solvent: ethyl acetate-butanone-formic acid-water, 50:30:10:10). When required a linearly rising methanol gradient was obtained by continually replacing the solvent leaving the solvent reservoir by pure methanol.

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1. Gelotte, B. *J. Chromatog.* **3** (1960) 330.
2. Bock, B. *Brewing Ind. Res. Inst., Illinois, Res. Notes No. 5* (1960), cited in Ref. 4.
3. Nilsson, A. *Acta Chem. Scand.* **16** (1962) 31.
4. Woof, J. B. and Pierce, J. C. *J. Chromatog.* **28** (1967) 94.
5. Somers, T. C. *Nature* **209** (1966) 368.

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## Studies on Synthetic Ascorbigen as a Source of Vitamin C for Guinea Pigs\*

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Ascorbigen is known to be a bound form of ascorbic acid chemically synthesized<sup>1</sup> or isolated from cabbage and other plants.<sup>2</sup> The question has arisen whether ascorbigen does act as a preventative and cure of scurvy as a source of vitamin C, since the native vitamin C in vegetables and plants is more stable and active than synthetic L-ascorbic acid in humans and guinea pigs.

Ascorbigen was synthesized chemically from ascorbic acid by conjugation with 3-hydroxymethylindole, which was prepared from 3-indolealdehyde and sodium borohydride, by the method of Virtanen and Kiesvaara.<sup>1</sup> The yield of pure ascorbigen, according to analyses by paper and thin-layer chromatography and estimation by ultraviolet absorption and infrared analysis, was approximately 67%.

Guinea pigs of a pure albino strain, weighing about 200 to 250 g, were used in the animal experiments. Twenty-four animals were fed on the scorbutic diet and tap water was provided *ad libitum*. On the 10th day of feeding, the animals were divided into four groups as follows: (1) control group on the scorbutic diet, (2) ascorbic acid group (2 mg per day), (3) ascorbigen group (3.5 mg per day), and (4) ascorbigen group (21 mg per day). Each group comprised three female and three male guinea pigs.

The animals in the control group were given 1 ml of 50% sucrose solution *per os* by pipette daily, and those in groups 3 and 4 were given supplements containing 3.5 and 21 mg ascorbigen, respectively. The animals in group 2 were given 2 mg of ascorbic acid dissolved in 1 ml of 50% sucrose solution. The animals were

\* Summarized from *Reports of the National Research Institute of Police Science* **18** (1965) 26. and body weight, appetite, movement, and

observed during 39 days of the experiment appearance were carefully recorded. The results obtained are summarized in Table 1, indicating the average body weight changes per day and the content of free and bound ascorbic acids in the whole liver homogenates of the guinea pigs.

Table 1. Average body weight changes per day and content of free and bound ascorbic acids in the liver of guinea pigs fed on a scorbutic diet (group 1), ascorbic acid (group 2) and ascorbigen (groups 3 and 4).

Group	Average body weight change per day (g)	Ascorbic acid in liver (mg)	
		Free	Bound
1	-5.4	0.25	0.024
2	+3.0	1.60	0.110
3	+0.16	0.31	0.027
4	+3.0	0.71	0.076

As is shown in Table 1, the weight of the animals in the control group decreased continuously at an average rate of 5.4 g per day after the 12th day of the experiment. Typical symptoms of scurvy were clearly seen between the 14th and 16th days: lack of appetite, apathy, and sore joints. Four animals died on the 23rd day after feeding on the scorbutic diet. In group 3, the weight of the animals increased at an average rate of 0.16 g per day, but two of them showed clear symptoms of scurvy and died on the 24th and 29th days. Meanwhile in groups 2 and 4 no symptoms of scurvy could be observed in the animals, whose weight increased at the same rate of 3.0 g per day on an average. In parallel with the increase of the body weight, the contents of the free and bound forms of ascorbic acid in the whole liver homogenates increased, but more free and bound ascorbic acid accumulated in the animals fed on 2 mg ascorbic acid per day than in those fed on 21 mg of ascorbigen, as is shown in Table 1.

According to these results, guinea pigs fed on ascorbigen were able to utilize only 15 to 20 % of the ascorbic acid bound in ascorbigen as a source of vitamin C. This

is in agreement with the findings of Kiesvaara and Virtanen.<sup>3</sup>

1. Virtanen, A. I. and Kiesvaara, M. *Acta Chem. Scand.* **17** (1963) 848.
2. Malaker, M. C. and Guha, B. C. *J. Indian Chem.* **38** (1961) 939; Prochazka, Z. and Senda, V. *Collection Czech. Chem. Commun.* **25** (1960) 270, 1100.
3. Kiesvaara, M. and Virtanen, A. I. *Acta Chem. Scand.* **16** (1962) 510; **17** (1963) 849.

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## A Gas Chromatographic Method for Determination of the Rancidity of Herring Oil

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As with other oils and fats, the rancidity of the oil of semi-sterile herring preserves is generally measured by determination of the peroxide number, carbonyl number, thiobarbituric acid (TBA) number, and so on. In the main, these numbers increase at the beginning of rancidity, but diminish again after some time. Consequently their application for evaluation of the quality of a mature product is limited.

A method has been published by Scholz and Ptak<sup>1</sup> for gas-chromatographic measurement of the rancidity of vegetable oils. They detected pentane in cottonseed oil, peanut oil, corn oil, and soybean oil, and further demonstrated the correlation of organoleptic quality and the content of pentane. It has been known for many years that pentane and other hydrocarbons are present in rancid oils. Buttery *et al.*<sup>2</sup> have suggested that hexanal is the source of saturated hydrocarbons C<sub>1</sub>-C<sub>5</sub>, and that their production is catalysed by fat peroxides.