aminobutyric acid (I) and of O-dithiocarbomethoxy-D-(−)-β-hydroxybutyric acid (II) have positive Cotton effects centered around the 330 and 350 mμ absorption bands, and the dispersion curves of N-dithiocarbomethoxy-L-(+)-β-amino-β-phenylpropionic acid (III) and of O-dithiocarbomethoxy-L-(+)-β-hydroxy-β-phenylpropionic acid (IV) have negative Cotton effects. Although the study of this problem has so far been limited to the investigation of only four structurally varied compounds, it seems as though the sign of the Cotton effect would indicate the absolute configuration of the β-asymmetric center in β-amino and β-hydroxy acids. Since, however, the derivatives of the α- and β-substituted acids of the same configuration have opposite sign of the Cotton effect, the following extension of the previously established relationship [1] is suggested: *If a dithiocarbamate of an amino acid, or a xanthate of a hydroxy acid, has an optical rotatory dispersion curve with a positive Cotton effect, then in the case of an α-amino or an α-hydroxy acid we have the* L*-configuration and in the case of a β-amino or a β-hydroxy acid the* D*-configuration.*

A full report of this work will be published later.

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Separation of α- and β-Glucometasaccharinic Acids by Means of Anion Exchange Chromatography

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In previous papers [1-3] it has been shown that hydroxy acids which are formed during the alkaline degradation of cellulose can be separated chromatographically by means of anion exchange resins. Several hydroxy acids containing 4−6 carbon atoms can be effectively separated by elution with borate solution, whereas with acids such as glycolic and lactic acid the separation is simpler and more efficient in acetate medium [4]. In connection with further work on the separation and identification of hydroxy acids which are formed in various technical processes, it has been found desirable to investigate the possibility of separating the stereoisomeric forms of glucometasaccharinic acids by means of anion exchange chromatography.

As shown by Kenner and Richards [5], β-glucometasaccharinic acid can easily be obtained in a pure form by alkaline degradation of 3-*O*-methylglucose. Large amounts of α-glucometasaccharinic acid are also formed in this reaction. In the present work (Figs. 1 and 3) it is shown that these stereoisomers can be effectively separated by chromatographic elution with 0.07 M sodium tetraborate solution under conditions which have been described in earlier papers for the separation of other saccharinic acids. The fractions were analyzed using the Technicon Auto-Analyzer [6] which means a considerable saving in time compared with the manual dichromate titration used earlier. The elution band corresponding to β-glucometasaccharinic acid appears before that of α-glucometasaccharinic acid and a quantitative separation can be obtained on a fairly short column.

Alkaline degradation of 3-O-methylglucose. The alkaline degradation of 3-*O*-methylglucose and the isolation of β-glucometasaccharinic acid from the reaction mixture has, with small changes, been performed according to the procedure given by Kenner and Richards [5]. A solution of 5.13 g of 3-*O*-methylglucose in

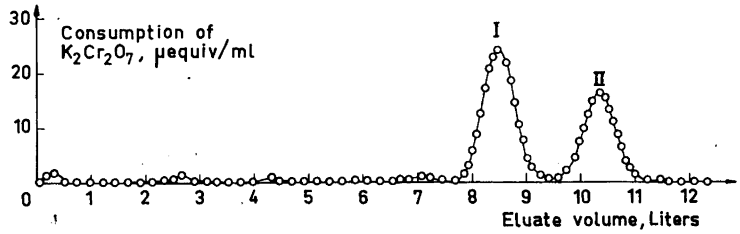

Fig. 1. Chromatographic analysis of the crude salt containing α- and β-glucometasaccharinic acids. (Found: C 35.8; H 5.7. Calc. for $(C_6H_{11}O_6)_2Ca$: C 36.2; H 5.6.
Column: 33 × 332 mm; Dowex 1 X—8 (borate form < 400 mesh). Flow rate: 0.3 ml $cm^{-2}min^{-1}$.

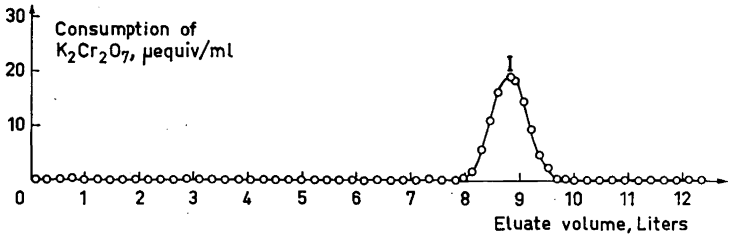

Fig. 2. Chromatographic analysis of β-glucometasaccharinic acid prepared according to Kenner and Richards [5].
Column: 33 × 332 mm; Dowex 1 X—8 (borate form < 400 mesh). Flow rate: 0.3 ml $cm^{-2}min^{-1}$.

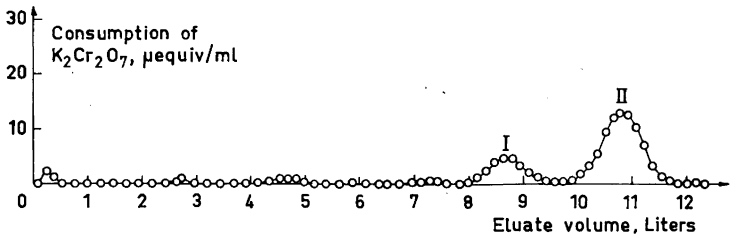

Fig. 3. Chromatographic analysis of the precipitate containing α- and β-glucometasaccharinic acids.
Column: 33 × 332 mm; Dower 1 X—8 (borate form < 400 mesh). Flow rate: 0.3 ml $cm^{-2}min^{-1}$.

1.2 l of oxygen-free lime water (0.062 N) was kept at 29°C for 192 h. The calcium ions were exchanged for hydrogen ions using a cation exchange resin. After washing with water, the effluent was evaporated at 40°C to a syrup which was dissolved in 50 ml of water. After treatment with charcoal, neutralization with calcium carbonate, and filtration, the solution was again evaporated to dryness. After trituration with ethanol, the crude calcium salt was obtained as a yellow crystalline powder (dry weight 5.04 g).

The results from the chromatographic separation (Fig. 1) show that two main fractions have been obtained. The chromatogram reveals traces of some other products. The position of the first elution peak (I) (main fraction) corresponds to that obtained with pure β-glucometasaccharinic acid isolated from the crude fraction according to the method given by Kenner and Richards (Fig. 2). According to these authors, the other main fraction obtained in this synthesis is α-glucometasaccharinic acid and it can therefore be assumed that elution band II contains this acid.

The sodium tetraborate was removed from these fractions by the same method as used in previous work [7]. In a paper chromatographic study (ethyl acetate-acetic acid-water [8,2]) the spots were located using permanganate-metaperiodate. Two distinct spots were obtained for each of the main fractions. The spots obtained from elution band I were identical with those obtained with the authentic β-glucometasaccharinic acid. The slower travelling spot on the paper chromatogram obtained with fraction II was identical with those obtained with fraction I and with authentic β-glucometasaccharinic acid, whereas the faster travelling spot had travelled a longer distance than the corresponding spots obtained with fraction I and with the authentic β-glucometasaccharinic acid. As found by Machell and Richards [9], the α-form of glucometasaccharinic acid travels ahead of the β-form, a fact which further supports the assumption that band II contains α-glucometasaccharinic acid.

The strychnine salt prepared from elution band I and recrystallized from ethanol, sintered and decomposed at 180—185°. The authentic sample of β-glucometasaccharinic acid exhibited the same behavior. No melting point depression was observed.

The brucine salt was prepared from elution band II and was recrystallized from abs. ethanol with slow cooling. The m.p. was 144—147°. Nef [10] reported m.p. 145—150° for brucine α-glucometasaccharinate, whereas Kenner and Richards [5] reported sintering and decomposition at 147—150°.

All these results indicate that elution band I corresponds to β-glucometasaccharinic acid and band II to α-glucometasaccharinic acid. From the areas under the elution curves the amounts of the acids present can be calculated. The ratio between the amounts of α-glucometasaccharinic acid and β-glucometasaccharinic acid was found to be 0.68.

Calcium β-glucometasaccharinate has been isolated from the crude calcium salt obtained by degradation of 3-*O*-methylglucose. The crude calcium salt was dissolved in hot water and the solution kept in a refrigerator at 3—4° for two days. Cubic crystals of calcium β-glucometasaccharinate were formed. The yield was 38 % of the crude salt. Ion exchange chromatography showed that only one elution band was obtained (Fig. 2). Its peak elution volume was the same as that obtained with an earlier work [1].

The mother liquor was treated with ethanol and the precipitated calcium salt, which according to Kenner and Richards [5] should contain a mixture of α- and β-glucometasaccharinic acids, was investigated chromatographically. The results given in Fig. 3 show that two main fractions are obtained corresponding to these two acids. As could be expected, the relative amount of α-glucometasaccharinic acid is much higher in this preparation than in the crude salt.

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