

phosphate dehydrogenase to generate NADPH. This was done by incubating with and without added enzyme under the same conditions as (d) and measuring partly the amount of NADPH formed during an incubation period, partly whether the enzyme could continue to form NADPH after the primary incubation. NADPH concentrations were determined by spectrophotometry at  $\lambda = 338 \text{ m}\mu$ .

Results are shown in Table 1. Apparently addition of citrate to the incubation medium not only stimulates total synthesis but also specifically enhances the synthesis of myristic acid. Compared with the results from synthesis without citrate stimulation (b), the percentage of total incorporated  $^{14}\text{C}$  activity found in myristic acid is 34 % higher when liver homogenates are incubated together with citrate (c). This difference can be shown to be statistically significant at the level  $p < 0.001$  when it is tested against a pooled standard deviation with  $6 \times (5 + 3 + 1) = 54$  degrees of freedom. A comparison between the results from the medium (b) and the results from incubations in a medium (d) containing another NADPH generating system than the one based on citrate, shows no enhancement of myristic acid synthesis. In the latter case the relative percentage of  $^{14}\text{C}$  activity found in myristic acid rather resembles the small values seen after synthesis by the liver slices. When the shown percentages do not add up to 100 %, the rest consisted mainly of a saturated  $\text{C}_{15}$  acid together with small amounts of a double-unsaturated  $\text{C}_{18}$  acid.

The observed citrate effect should be viewed in relation to the general problem of what makes the *de novo* synthesis of saturated fatty acids normally stop at the palmitic stage. A closer examination of the mechanism of citrate stimulation may help to clarify this matter. In view of the findings by Lorch, Abraham and Chaikoff<sup>5</sup> it could seem at present as if malonyl-CoA generated by citrate, in contrast to artificially synthesized malonyl- $^{14}\text{C}$ -CoA, had difficulties in elongating myristic acid to palmitic acid. Our results show that the relative myristic enhancement is not due to stimulation by NADPH.

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## A New Sennoside from *Cassia* Species

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The extensive studies by Stoll *et al.*<sup>1</sup> on anthrone glycosides of *Senna* species resulted in the isolation and characterization of two isomeric sennosides, the optically active (+),(+)-bis-rheinanthrone-8-glycoside or sennoside A and the intramolecularly compensated mesoform (+),(−)-bis-rheinanthrone-8-glycoside or sennoside B. We have now isolated a new sennoside from *Cassia* species. The name sennoside C has already been given to another compound.<sup>2,3</sup> Therefore, the name sennoside III is proposed for the new substance.

For the isolation of sennoside III finely ground leaves of *Cassia acutifolia* or *Cassia angustifolia* are defatted by extraction with chloroform. The fat-free leaves can then be extracted with any of the conventional solvents previously used for isolation of sennosides A and B. However, the best results have been obtained by using glacial acetic acid as extractant; the sennosides,

as naturally occurring salts, are readily soluble in this solvent and can easily be precipitated by addition of ether.

The mixture of sennosides obtained is dissolved in water and treated with Amberlite IR-120-H<sup>+</sup> resin to liberate the free sennosides. The bulk of sennoside A is allowed to crystallize from the acid solution and is filtered off. The filtrate is fractionated by liquid-liquid extraction. The solvent mixture is prepared by mixing methyl-ethyl ketone (50), acetic acid (1) and water (30) and the distribution coefficients are: sennoside A 0.52, sennoside B 0.22, sennoside III 0.11, and rhein-anthroneglycoside 1.6.

The combined fractions containing the third sennoside are evaporated to dryness in vacuum at a temperature not exceeding 40° and the residue is dissolved in ethyl alcohol. Precipitation of sennoside III is obtained by addition of ether. The yield of crude sennoside III is about 0.15 %.

Previously published chromatographic methods for the separation of sennosides A and B<sup>4-7</sup> were found to be useless for the separation of sennoside III, which by all these methods showed an equal  $R_F$  value to sennoside B. The three substances could be separated by paper chromatography, however, using the upper layer obtained by mixing methyl-ethyl ketone (300), acetic acid (1.5) and water (150) and allowing the elution to continue for 16–18 h. The  $R_{FA}$  values, when sennoside A is taken as 1, are: sennoside B 0.63, and sennoside III 0.50.

Sennoside III can also be separated from the other sennosides by thin-layer chromatography on polyamide in methanol-propionic acid (25:1). The approximate  $R_F$  values in this system are: sennoside A 0.21, sennoside B 0.57, and sennoside III 0.75.

Sennoside III is a very easily decomposing compound which, in spite of repeated attempts, has not been obtained in crystalline form. It is very soluble in water, soluble in glacial acetic acid and the lower alcohols, slightly soluble in acetone and practically insoluble in chloroform, ether, benzene and hexane. For the following studies of the properties of sennoside III, repeatedly precipitated, slightly yellowish or brownish samples melting unsharply between 220 and 240° have been used.

*Hydrolysis of sennoside III.* In the quantitative determination of the sugar liberated by acid hydrolysis 1.8 moles of monosaccharide (as glucose) calculated on the basis of the

proposed formula, was obtained. Simultaneously performed determinations on sennosides A and B yielded 1.9 and 1.8 moles of sugar, respectively.

Even after chromatography in three solvent systems only one spot corresponding to glucose was observed.

After hydrolysis and oxidation sennoside III gave a positive Bornträger reaction similar to sennosides A and B.<sup>8</sup>

*Spectral data of sennoside III.* The ultraviolet spectrum of sennoside III in 0.1 M sodium bicarbonate showed maxima at 348, 308, and 268  $\mu$ . The location and intensity of the maxima are practically identical with the corresponding data of sennoside B.

The major bands of the infrared spectrum of sennoside III were identical with those of sennosides A and B.

*Reduction and oxidation experiments.* Paper chromatography of the reduction product of sennoside III resulted in one major spot identical with rheinanthrone-8-glycoside obtained by reducing sennosides A or B under similar conditions.<sup>1</sup>

When rheinanthrone-8-glycoside was oxidized by air in the presence of palladium as catalyst,<sup>1</sup> besides sennosides A and B small amounts of sennoside III were identified and isolated from the mixture by the liquid-liquid extraction method.

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The present paper is a preliminary report on this subject of which full details are being published in the near future.

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