co-workers initially proposed[3] the structure of dihydropriverogenin A possessing 22β-hydroxyl function (axial) (Ⅲ), while very recently camelliagenin A was assigned with 22α-hydoxyl (equatorial).[5] We examined the nuclear magnetic resonance spectra of dihydropriverogenin A triacetate (Ⅳ) and 22-epimeric triacetate (Ⅴ) in relation to the acetyl derivatives of theasapogenols[6] and found that dihydropriverogenin A (=camelliagenin A) should be expressed by Ⅱ, which will be discussed in detail elsewhere from our laboratory.[7]

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A New Colorimetric Method of Sodium Cyclamate by Solvent Extraction with Tris(1,10-Phenanthroline)iron^{+2} Chelate*1,2

Cyclamate has been commercially available as a synthetic sweetening agent since 1950. Common method for determination of cyclamate involves titrimetry, gravimetry and gas chromatography. However, relatively few colorimetric determinations have been applied to food and drugs because the procedures are tedious and usually do not give a very accurate result, as pointed out in the review described recently by M. L. Richardson.[1]

It is the purpose of this investigation to make a new colorimetric procedure in determination of cyclamate which includes a separation treatment from saccharin sodium using a molecular sieve column.

It occurs to the authors that a small amount of cyclamate in an aqueous solution is selectively extracted into nitrobenzene if a considerable amount of tris(1,10-phenanthroline)iron^{+2} sulfate is added, and that there is a linear relationship between the red color intensity of the organic phase and the amount of cyclamate present. Fig. 1 shows the visible absorption spectra in the organic phase. It can be observed that the tris(1,

*1 Part XXIX in the series entitled "The Spectrophotometric Determination of Anions by Solvent Extraction with Metal Chelate Cations." Part XXVIII. K. Hiiro, T. Tanaka, Y. Yamamoto: Bunseki Kagaku, to be published.
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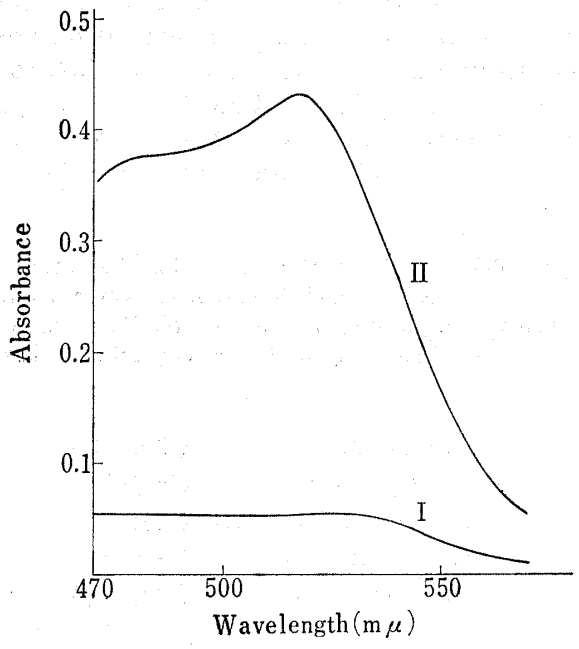

Fig. 1. Absorption Spectra

Curve I : cyclamate absent
Curve II : cyclamate present ($8\times10^{-4}M$)
Reference : nitrobenzene

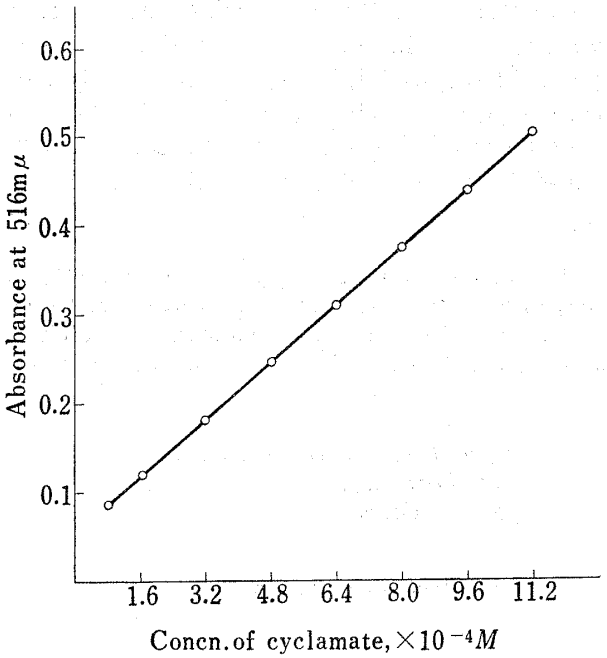

Fig. 2. Calibration Curve

10-phenanthroline)iron^{+2} cation is readily extracted into nitrobenzene when a small amount of cyclamate is present in the aqueous phase. The maximum absorbance of the extracted species in nitrobenzene occurs at a wavelength of 516 mμ. The chemical formula of the extracted species is assumed to be $[Fe(phen)_3\cdot Cy_2]$, where phen is represented as 1,10-phenathroline and Cy is as cyclamate anion. A maximum extraction is obtained in the pH range 3~12, when an excess of at least 7-fold (molar) of the phenanthroline chelate to cyclamate is present. The color intensity of the extracted species remains constant at room temperature for several hours after the separation of the organic layer. A recommended procedure for the calibration curve is as follows.

Three ml. of tris(1,10-phenanthroline)iron^{+2} sulfate solution ($5\times10^{-2}M$), 5 ml. of phosphate buffer solution (0.1*M*, pH 5~7) and varying amounts (0.5~8.0 ml.) of the standard cyclamate solution ($4.0\times10^{-3}M$) are mixed. Then the mixture is diluted to 25 ml. with distilled water, and it is shaken for 2~3 min. with 10 ml. of nitrobenzene. After standing the mixture for about 30 min., the organic layer is transferred to a test tube containing 1 g. of anhydrous sodium sulfate and it is shaken in order to make the solution transparent. The absorbance of the extracted solution is measured at 516 mμ using 10 mm. glass cells against a reagent blank solution as a reference. A linear relationship is obtained over the tested range of 8×10^{-5} to $1\times10^{-3}M$ of cyclamate in aqueous solution, as shown in Fig. 2.

Interference study has resulted that a large amount of other sweentening agent such as dulcin, succharose, glucose and lactose has almost no effect on the absorbance even when present in molar quantity several hundred times that of cyclamate. However, saccharin is found to seriously interfere with the determination of cyclamate. For example, concentrations greater than 1 p.p.m. of added saccharin sodium give rise to a relative error greater than 6% for cyclamate at the 160 p.p.m. level. Many inorganic salts such as phosphate, sulfate and borate, when present in moderate concentrations, cause no interference. Chloride gives a positive error when present in p.p.m. concentration 2-fold that of cyclamate.

Table I. Determination of Sodium Cyclamate in Synthetic Sweetening Mixtures

Sample No.	Sodium Cyclamate (p.p.m.)		Recovery (%)
	Taken	Found	
1	160	160	100
2	160	176	110
3	65	187	288
4	128	128	100

No. 1 cyclamate
No. 2 cyclamate+chloride (300 p.p.m.)
No. 3 cyclamate+saccharin (50 p.p.m.)+dulcin (50 p.p.m.): separation by the offical method
No. 4 cyclamate+saccharin (154 p.p.m.)+dulcin (115 p.p.m.): separation by the present method

In applying the proposed analytical method for a sample such as an artificial sweetners preparation (saccharin sodium, sodium cyclamate and dulcin mixture), it is necessary to remove saccharin from the mixture. Although cyclamate can be quantitatively separated from saccharin according to the official method[2] using anion exchange resin column, a considerable positive error is observed on the determination of cyclamate (Table I, Sample No. 3). The higher recovery of cyclamate may be attributed to the presence of a large amount of chloride necessarily contained in the eluent solution (0.1*N* HCl). It is, therefore, desirable to expel the chloride in the eluent, or to use another method of the separation. In our study, it is found that a quantitative recovery is obtained if the separation is done with a molecular sieve column (Sephadex G-10) using phosphate solution as eluent (Table I, Sample No. 4). The procedure of separation is as follows.

Sephadex G-10 swelled by 0.05*M* phosphate buffer solution (pH 5~7) is filled in a glass cylinder of 1 cm. diameter so that the column height may be 30 cm. Two ml. of mixed sample solution placed on the top of the column is eluted by the phosphate buffer solution (0.05*M*, pH 5~7). The first fraction of the eluent is received to the mark in a 50 ml. measuring flask, where the elution velocity is kept at 1 ml. per 3 min. An aliquot of this solution is pipetted out and the recommended procedure for the determination of cyclamate, described above, is to be applied.

Several features of the proposed method are as follows. The reagents employed are readily available in pure form, and their solutions remain stable over long periods of storage. The method is sensitive, accurate and highly selective for the detection and determination of cyclamate, and will be useful for analysis of cyclamate contained in food or drug preparations.

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