Notes

Preparation of High Purity Xylenol Orange and Isolation of a New Metallochromic Dye by Cation Exchange Column Chromatography on SP-Sephadex

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Xylenol Orange (XO), 3,3'-bis[*N,N*-bis(carboxymethyl)aminomethyl]-*o*-cresolsulfonphthalein, has been known as the most useful sulfonphthalein complexone metallochromic indicator.^{1,2} It has been extensively studied in its metal complex formation equilibria.^{3,4} To obtain pure XO from its crude form, however, is not easy.^{5,6} For example, even after being purified by cellulose column chromatography, there still remains several % of an unidentified colored impurity (β -XO).⁷

The present paper describes the synthetic condition to obtain XO of high purity, or to minimize the formation of byproducts such as Semi-XO (SXO) and β -XO, in place of separating them. It also describes the isolation of byproducts having a very analogous structure with XO, i.e., β -XO and a new metallochromic dye, Glycine Iminodiacetic acid Cresol Red (GICR), 3-[*N*-(carboxymethyl)aminomethyl]-3'-[*N,N*-bis(carboxymethyl)aminomethyl]-*o*-cresolsulfonphthalein, by using cation exchange column chromatography on SP-Sephadex under an acidic condition which was developed for analyzing synthetic reaction products. The chromatography was so efficient in separating many colored materials within a short time that it was quite suitable for a ready and speedy purification of commercial sulfonphthalein dyes.

Fig. 1 Structure of GICR and the numbering of carbons.

Experimental

All the reagents and procedures were the same as those described previously⁸, except for the measurement of the ¹³C-NMR spectra, which was performed in D₂O solutions at various pD with a JEOL JNM-FX-100/PFT-100 spectrometer. Dioxane ($\delta=67.4$) was used as an internal reference.³

Synthesis of Xylenol Orange

A mixture of Cresol Red (CR; 1.91 g, 5 mmol), iminodiacetic acid (IDA; 2.66 g, 20 mmol), anhydrous sodium acetate (5.10 g, 62 mmol) and acetic acid (50 ml) was kept at 55°C, and 2.5 ml of aqueous 37% formaldehyde (31 mmol) were added to it. The mixture was kept at 55°C under stirring for 2.5 h, and then cooled in an ice bath and left overnight at room temperature. A supernatant of the mixture was used for further treatment.

Column chromatography on SP-Sephadex

Synthetic solutions in acetic acid (0.5 ml) were developed on 23 mm i.d.×220 mm or 90 mm H-form SP-Sephadex columns with 50 mM HCl for product analysis.⁹ For preparative purpose the following procedure was carried out. To a synthetic mixture diethylether was added in order to precipitate crude XO.⁸ The obtained precipitate was dissolved in 1 l of water; the mixture was then poured into a 28 mm i.d.×22 mm Cl-form DEAE-Sephadex column, washed with water and eluted with 100 mM HCl. The effluent was dried *in vacuo*. The residue was dissolved in a small amount of 50 mM HCl and the mixture developed on a 23 mm i.d.×220 mm SP-Sephadex column with 50 mM HCl.

Glycine Iminodiacetic Acid Cresol Red (GICR) and β -XO

GICR was collected from the effluent of preparative

chromatography of synthesized XO on SP-Sephadex. The effluent was diluted with water ten times, and was then poured into a 22 mm i.d.×30 mm DEAE-Sephadex column. The column was washed with 60 ml of water, developed with 50 mM NaCl and then with 100 mM NaCl. The fraction of GICR⁹ was then diluted with water, adsorbed to DEAE-Sephadex, and eluted with 100 mM HCl. The effluent was filtered and dried *in vacuo*. ¹³C-NMR [D₂O, pD 1.6] (see Fig. 1): δ 173.8, 173.4 (C-4, 4'), 169.0 (C-10, 10'), 166.0 (C-0), 142.7 (C-12), 140.9 (C-6, 6'), 139.2 (C-2, 2'), 137.3 (C-11), 132.5 (C-15), 131.6 (C-14), 131.3 (C-16), 131.1 (C-5, 5'), 130.5 (C-1, 1'), 128.9 (C-13), 123.3, 120.7 (C-3, 3'), 56.0 (C-8), 55.2 (C-9), 42.5 (C-8'), 47.9 (C-9'), 16.6 (C-7, 7') ppm.

β -XO was collected⁹ from the effluent of SP-Sephadex chromatography of crude XO synthesized under Korbl's conditions.^{1,5} It was then purified using the same procedure as described for GICR. β -XO was eluted in front of GICR in the aforementioned DEAE chromatography. ¹³C NMR [D₂O, pD 1.6]: δ 210.9 (C-17), 173.3, 172.2 (C-4, 4'), 169.7, 169.0 (C-10, 10'), 166.0 (C-0), 142.6 (C-12), 140.9, 139.8 (C-6, 6'), 139.2, 138.8 (C-2, 2'), 137.4 (C-11), 132.5 (C-15), 131.7 (C-14), 131.3 (C-16), 131.1 (C-5, 5'), 130.6, 130.5 (C-1, 1'), 128.8 (C-13), 123.3, 120.7 (C-3, 3'), 56.1 (C-8), 53.3 (C-9), 47.6 (C-8'), 47.9 (C-9'), 46.4 (C-7, 7') ppm.

Results and Discussion

Synthetic condition to obtain XO of high purity

Using SP-Sephadex column chromatography for product analysis, synthetic conditions of XO were investigated. The best one is described in the experimental section. Compared to conventional method^{1,5}, the reaction period was shorter (2.5 h compared to 10 h), the reaction temperature lower (55°C compared to 65°C), and had a two-fold higher concentrations of IDA, sodium acetate, and formaldehyde. The synthetic yield was improved from 80% to 98% and most of the byproducts decreased to undetectable amounts with very little amounts of SXO (1.2%) and GICR (0.8%).

Byproducts of XO synthesis; new metallochromic dyes

When the synthetic solution obtained under the present synthetic condition was chromatographed on a SP-Sephadex column, several minor colored bands appeared in addition to the two main bands of XO and SXO (Fig. 2). In order to find new types of complexone dyes among the byproducts, the properties of two kinds of the main byproduct dyes were investigated.

(1) Glycine Iminodiacetic Acid Cresol Red (GICR).

The dye of a yellow band next to the XO band in the elution curve (Fig. 2) showed a color change to violet ($\lambda_{\max}$ =570 nm) upon adding Cu^{II} at pH 5. The ratio of maximum absorption of the Cu^{II} complex at 570 nm to that of free dye at 433 nm was 2.0. The color change

Fig. 2 Chromatogram of a synthetic solution of XO from an SP-Sephadex cation exchange column: eluent, 50 mM HCl. Arrows indicate yellow bands of unidentified materials.

was similar to that observed for Glycine CR and Semi-GICR. The dye changed color to reddish brown with a large excess of Fe^{III} in 50 mM HCl. The absorption spectrum of the solution had a maximum at 488 nm and two shoulders at 550 and 600 nm. The shoulder at 550 nm corresponded to the absorption maximum ($\lambda_{\max}$) of the Fe^{III}-SXO complex (548 nm). Thus, the dye was suggested to have both IDA and glycine moieties. To confirm this assignment, a synthetic reaction was performed by adding 2 mmol of glycine to the reactants of the present XO synthesis (5 mmol CR and 20 mmol IDA). The yield of the dye increased drastically from 0.8% to 17%, indicating that it is a cross Mannich condensation product of glycine and IDA with CR. The glycine may have been produced by the hydrolysis of IDA.

The ¹³C-NMR spectra of the dye was measured in D₂O at various pD in order to confirm its structure (for the NMR data, see experimental section). It was quite similar to that of XO³, except that some of the signals split into two, depending on pD; also, it had two more signals at 42.5 and 47.9 ppm (values at pD 1.6). The two signals can be assigned to those of aminomethylene (C-8', see Fig. 1) and carboxymethylene (C-9') carbons of glycine moiety (the signals of IDA moiety were at 56.0 (C-8) and 55.2 (C-9) ppm). The splitting of some signals reflected the inequivalence of A- and B-rings (Fig. 1) caused by cross Mannich condensation. Thus, the NMR results also indicated that the byproduct is GICR.

(2) β -XO. The dye of a yellow band next to the GICR band in the elution curve was produced when XO was synthesized under Korbl's conditions with a yield of several percent. The yield increased for a longer reaction time and/or higher temperature.⁷ The unidentified dye was named β -XO by Yamada *et al.*, since it had almost an equivalent molecular formula to XO, though different from XO regarding the color of the Fe^{III} complex.⁷ Its spectrum with large excess of Fe^{III} in 50 mM HCl showed an absorption maximum at 479 nm and a shoulder at 550 nm, the latter corre-

sponding to λ_{max} (548 nm) of Fe^{III} -SXO, suggesting that β -XO has only one IDA moiety. The color of β -XO did not change much with a large excess of Cu^{II} at pH 4.8.

The ^{13}C -NMR spectra of β -XO were close to those of GICR. Some of the signals split into two, indicating an inequivalence of the A- and B-rings. It had two high field signals at 47.6 and 47.9 ppm, showing the presence of a moiety other than the IDA moiety. The most striking feature of the spectra was the presence of an unusually low field signal around 210 ppm. The profile of the NMR spectra may suggest that β -XO has a triketone moiety formed by a ring condensation of the IDA moiety and formaldehyde as:

Then, the signal at 210 ppm can be assigned to the central carbon (C-17) of the triketone¹⁰ and the signals at 47.6 and 47.9 ppm to aminomethylene (C-8') and carbonylmethylene (C-9') carbons of the moiety.

Cation exchange column chromatography on SP-Sephadex under acidic condition

As shown in Fig. 2, many colored materials were readily separated by the column method. The elution order of reactants and products of XO synthesis was $\text{IDA} < \beta\text{-XO} < \text{GICR} < \text{XO} < \text{SXO} < \text{CR}$, reflecting the difference in their mean charges (1, 0 and -1) in a 50 mM HCl eluent. The small charges under the acidic condition allowed for a speedy occurrence of the cation exchange chromatography.¹²

Purification of commercial dyes. Since commercial XO's still contain considerable amounts of SXO and other impurities¹¹, it is preferable to purify them before use in order to obtain highly reliable results in spectrophotometry, or a sharp color change at the end point in chelatometry. A column chromatography on SP-Sephadex is quite suitable for this purpose; commercial dye was dissolved in a small amount of 50 mM HCl, loaded on

a column of 90-mm length and was eluted with 50 mM of HCl. The separation of SXO and XO was achieved within 10 min. In order to obtain pure XO, top and tail parts of the XO band should be eliminated, the parts of which contained GICR and β -XO. The method was not, however, appropriate for isolating pure XO, since it was difficult to remove Cl^- and a sulfur-containing impurity (probably sulfoxypropyl sugars) from the dye. Methylthymol Blue and Methylxlenol Blue were also purified readily by using a column 220 mm in length with 10 mM HCl eluent.¹²

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