Short Communication

A New Scalable Route to Resolving the α -Monoesters of dl 5-Formyl 5,6,7,8-Tetrahydropteroylmono-L-Glutamic Acid

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Introduction

Recently, we reported a novel, wholly synthetic route to dl 5-formyl tetrahydropteroylpoly- γ -L-glutamic acid derivatives (1). This process is potentially employable in the synthesis of nearly all known tetrahydropteroylpoly-L-glutamate derivatives. The key to our method is the preparation of an α -2,6-dichlorobenzyl monoester of dl 5-formyl tetrahydropteroylmono-L-glutamic acid. While purifying the α -monoester on a silica gel column, we observed its partial separation into d-L and l-L diastereomers. A review of the literature disclosed that all previously reported chromatographic resolutions of a tetrahydropteroylmono-L-glutamic acid derivative required either a chiral derivatizing agent or a chiral stationary phase (2–6). To the author's knowledge, the method described herein represents the first example in which the resolution of such a mixture is achieved through achiral chromatographic means.

Methods

Chromatographic resolution of the α -2,6-dichlorobenzyl monoesters of d and l 5-formyl tetrahydropteroylmono-L-glutamic acid was performed with a Hewlett-Packard (Avondale Division, Avondale, PA, U. S. A.) liquid chromatograph system. The components included a model 1050 pumping system, a 1050 UV-vis variable wavelength detector set at 280 nm (0.02 to 2.0 a.u.f.s.), and a 3396A integrator. The HPLC column module (Dynamax-60A 8 μ m, 250 $\times$ 4.6 mm I.D.) and guard module (Dynamax-60A 8 μ m, 15 $\times$ 4.6 mm I.D.) were composed of spherical silica gel (Rainin Instrument Co., Inc. Woburn, MA, U. S. A.).

The calcium salts of dl and l 5-formyl tetrahydrofolate were the kind gift of Sapec S.A. Fine Chemicals (Lugano, Switzerland). The α -2,6-dichlorobenzyl esters of the dl and l 5-formyl tetrahydropteroylmono-L-glutamic acids were prepared as described previously. HPLC-grade chloroform and methanol were purchased from Baxter Diagnostics, Inc. (McGaw Park, IL, U. S. A.). 0.1 N NaOH was purchased from Fisher Scientific (Fair Lawn, NJ, U. S. A.) and glacial acetic acid from Mallinckrodt Specialty Chemicals Co. (Paris, KT, U. S. A.).

Achiral chromatography was performed under ambient conditions using an isocratic mobile phase of 95% chloroform and 5% methanol (v/v, the methanol phase contained 3% of glacial acetic acid). The flow rate was maintained at 1 ml/min.

Standard solutions (10.0 mg of sample per 2.5 ml of chloroform-methanol (5 : 1)) of the dl and l α -2,6-dichlorobenzyl monoesters of 5-formyl tetrahydropteroylmono-L-glutamic acids were prepared for analysis. Standard injection volumes ranged from 5 to 250 μ l.

Results

The standard elution profile of the α -2,6-dichlorobenzyl monoester of dl 5-formyl tetrahydropteroylmono-L-glutamic acid is shown in panel A. The two peaks represent the d-L (1, 48%) and l-L (2, 52%) diastereoisomers, respectively. The latter assignments were confirmed in two ways: (i) through comparison against a pure sample of the α -2,6-dichlorobenzyl monoester of l 5-formyl tetrahydropteroylmono-L-

glutamic acid (panel B), and (ii) by hydrolyzing the separated α -monoesters (10 minutes in 0.1 N NaOH solution at room temperature followed by neutralization with glacial acetic acid), then analyzing the solution on an albumin-linked silica gel column (7). In this manner, the assignments shown in panel A were firmly established.

Discussion

The method presented in this communication serves as a useful way of determining the chiral purity of α -monoesters of 5-formyl tetrahydropteroylmono-L-glutamic acid. Given the low cost of silica gel, experiments directed at the large-scale utilization of this procedure are underway.

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References

1. Fitzhugh, A. L., Chmurny, G. N. & Klose, J. R. (1991) *Bioorg. Med. Chem. Lett.* **1**, 155–158.
2. Kaufman, B. T., Donaldson, K. O. & Keresztesy, J. C. (1963) *J. Biol. Chem.* **238**, 1498–1500.
3. Hillcoat, B. L. & Blakley, R. L. (1964) *Biochem. Biophys. Res. Commun.* **15**, 303–307.
4. Matthews, R. G. & Haywood, B. J. (1979) *Biochemistry* **18**, 4845–4851.
5. Feeney, J., Albrand, J. P., Roberts, G. C. K., Burgen, A. S. V., Charlton, P. A. & Young, D. W. (1981) *Biochemistry* **20**, 1837–1842.
6. Rees, L., Valente, E., Suckling, C. J. & Wood, H. C. S. (1986) *Tetrahedron* **42**, 117–136.
7. Wainer, I. W. & Stiffen, R. M. (1988) *J. Chromatogr.* **424**, 158–162.