Direct Optical Resolution of ($\pm$)-Biotin and ($\pm$)-Epibiotin by a Reversed-Phase High-Performance Liquid Chromatography

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Summary A first and efficient direct optical resolution of biotin diastereomers, ($\pm$)-biotin and ($\pm$)-epibiotin, was accomplished by a reversed-phase high-performance liquid chromatography on cellulose tris(3,5-dimethylphenylcarbamate) coated on silica-gel.

Key Words biotin, vitamin H, epibiotin, high-performance liquid chromatography, optical resolution, cellulose tris(3,5-dimethylphenylcarbamate), reversed-phase, optical purity

(+)-Biotin is one of the water-soluble B vitamins and functions as a cofactor for enzymes principal to carboxylation reactions. Biotin contains three asymmetric carbon atoms; therefore there should exist eight diastereomers, ($\pm$)-biotin (1,2), ($\pm$)-epibiotin (3), ($\pm$)-allobiotin (2), and ($\pm$)-epiallobiotin (1,2). Industrially, (+)-biotin has been produced by a reaction sequence consisting of an optical resolution and an asymmetric hydrogenation (4). Thus, those diastereomers, especially (–)-biotin and ($\pm$)-epibiotin, could be contained as impurities in the synthesized (+)-biotin. While its enantiomeric purity has been determined based on the specific optical rotation, essentially such a method could not apply to diastereomers. Hence, the determination of the enantiomeric purities by direct separation of diastereomers has long been required. It has recently been demonstrated that high-performance liquid chromatography (HPLC) is suitable for the determination of ($\pm$)-biotin (5–8). However, to our knowledge, the optical resolution by HPLC has not been reported. This is mainly due to its unique structure containing three chiral centers and low solubility in eluting systems of normal phases which have been successfully applied to optical resolutions of various enantiomers. Recently, despite their relative scarcity, reversed-phase chiral stationary phases, such as ovomucoid bound on aminated silica-gel (ULTRON ES-OVM, Shinwa Chemical Industries, Ltd.), cellulose tris(3,5-dimethylphenylcarbamate) coated on silica-gel (CHIRALCEL OD-R, Daicel Chemical Industries, Ltd.), have been commercialized. As a preliminary experiment, the former was utilized to direct optical resolution of ($\pm$)-biotin; it, however, has proved unsuccessful. The

Fig. 1. (+)-Biotin and its analogs.

present study involves the use of reversed-phase HPLC on cellulose tris(3,5-dimethylphenylcarbamate) coated on silica-gel for the direct optical resolution of (±)-biotin and (±)-epibiotin to determine the optical purity of (+)-biotin in bulk.

MATERIALS AND METHODS

Materials. (±)-Biotin and (±)-epibiotin were synthesized as depicted in Fig. 2. The Wittig condensation of (±)-(3 α ,6 α)-1,3-dibenzyl-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one-4-triphenylphosphonium bromide [(±)-2], synthesized from (±)-(3 α ,4 α ,6 α)-1,3-dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(±)-1] (9), with methyl 4-formylbutyrate gave the (±)-5-[(3 α ,5 α)-1,3-dibenzyl-3a,4,6,6a-tetrahydro-2-oxo-4*H*-thieno[3,4-*d*]imidazol-4-ylidene]-pentanoic acid [(±)-3] as a major product. The assignment of (*E*)-geometry of (±)-3 was made by the observation of the nuclear Overhauser effect in ¹H-NMR between C(3a)-H and vinyl-H. The reduction of (±)-3 followed by debenzylation yielded (±)-biotin and (±)-epibiotin in a ratio of 77/23. This is in apparent contrast with the observation that the reduction of the (*Z*)-isomer of (±)-3 gave (±)-dibenzyl biotin and (±)-dibenzyl epibiotin in a ratio of 97/3. The two diastereomers ((±)-biotin and (±)-epibiotin) were separated by the preparative HPLC on Novapak C₁₈ (Waters, Japan Millipore, Ltd., 30×0.78 (ID) cm) by use of 0.05 M potassium dihydrogenphosphate (pH 2.5)-acetonitrile (65:35) as an eluting system. The structure of (±)-biotin was confirmed by the fact that its HPLC retention time and ¹H-NMR spectra were in agreement with those of (+)-biotin. The identification of (±)-epibiotin was performed based on the following data. mp. 191–194°C (Ref. (3): 190–191°C); ¹H-NMR (DMSO-*d*₆, 270 MHz) δ : 1.2–1.7 (6H, m, CH₂), 2.20 (2H, t, *J*=7.1 Hz, CH₂COOH), 2.58 (1H, dd, *J*=2.8 and 12.4 Hz, C(6)-H), 2.94 (1H, m, C(4)-H), 3.02 (1H, dd, *J*=5.6 and 12.4 Hz, C(6)-H), 3.86 (1H, dd, *J*=2.9 and 8.3 Hz, C(3a)-H), 4.29 (1H, m, C(6a)-H), 6.44 and 6.56 (each 1H, s, NH), 11.98 (1H, br s, COOH); IR (KBr) cm⁻¹: 3400, 1700, 1655, 1270; anal. calcd. for C₁₀H₁₆N₂O₃S: C, 49.16; H, 6.60; N, 11.47; S, 13.12. Found: C, 49.07; H, 6.47; N, 11.74; S, 13.28.

Fig. 2. Synthesis of ($\pm$)-biotin and ($\pm$)-epibiotin.

Chromatography. HPLC was carried out on a TOSOH Corporation CCPM chromatograph equipped with a Hitachi, Ltd., 655A-21 UV detector under the following conditions: column, CHIRALCEL OD-R (Daicel Chemical Industries, Ltd., 25 $\times$ 0.46 (ID) cm $\times$ 2 pieces); temperature, 40 $^{\circ}$ C; mobile phase, 0.05 M potassium dihydrogenphosphate (pH 2.5)-acetonitrile (90:10); flow rate, 0.4 ml/min; wavelength of detection, 220 nm.

RESULTS AND DISCUSSION

Four diastereomers, ($\pm$)-biotin and ($\pm$)-epibiotin, were resolved in good separation coefficient (α =1.17 for ($\pm$)-biotin and 1.09 for ($\pm$)-epibiotin) as shown in Fig. 3. The optical resolution by HPLC using chiral stationary phases is based on the formation of transient diastereomeric complex between enantiomers and chiral stationary phases. These diastereomeric complexes must differ adequately in free energy for effective optical resolutions (10). Considering such postulation, the exact position of polar groups which interact with chiral stationary phases, and of chiral centers would be crucial. It is noteworthy that C-4, which is the chiral center of biotin, separates from the carboxyl group in five carbons and two carbons from the ureide group. Furthermore, ($\pm$)-biotin and ($\pm$)-epibiotin do not have any aromatic group which could possibly induce a π - π interaction between diastereomers and the stationary phase, while the π - π interaction has been reported to be of importance, especially in reversed-phase systems (11). Hence the present findings demonstrate a broad applicability of this cellulose tris(3,5-dimethylphenylcarbamate) column.

In summary, ($\pm$)-biotin and ($\pm$)-epibiotin were optically resolved using the cellulose tris(3,5-dimethylphenylcarbamate) column; hence, the present method has the advantages of being accurate, fast, and direct, as it requires no derivatization. Application for this method will be not only the determination of enantiomeric purities of (+)-biotin on an analytical scale, but also the isolation on a preparative scale.

Fig. 3. Optical resolution of (±)-biotin and (±)-epibiotin by HPLC. Flow rate, 0.4 ml/min; temp., 40°C; detect., UV (220 nm); injection, 10 µl; (±)-biotin 1.3 mg/ml; (±)-epibiotin 1.3 mg/ml.

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