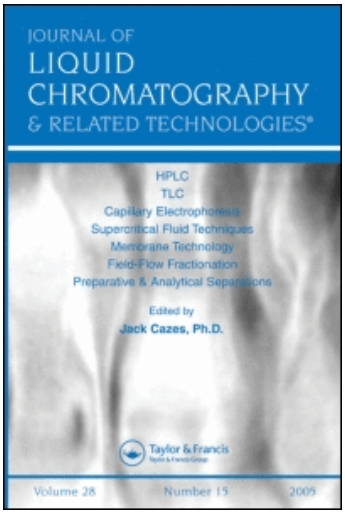

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Utility of Cyclodextrin in Mobile Phase for High-Performance Liquid Chromatographic Separation of C_{21} Steroids

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UTILITY OF CYCLODEXTRIN IN MOBILE PHASE FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF C_{21} STEROIDS

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ABSTRACT

The chromatographic behavior of C_{21} steroids, especially corticoids, and its fluorescent derivatives with anthroyl cyanide was examined by the reversed-phase high-performance liquid chromatography. The separation of the compounds was much improved by the addition of the suitable cyclodextrin to the mobile phase.

INTRODUCTION

In recent years considerable attention has been focused on inclusion chromatography using host-guest interaction in gas and liquid chromatography [1]. In a previous paper we reported the much improved separation of estrogens [2], cardiac steroids [3] and bile acids [4] by the addition of the suitable cyclodextrin

(CD) to the mobile phase in reversed-phase high-performance liquid chromatography (HPLC). As a continuation of this work, the present paper deals with the application of this method to the separation of C_{21} steroids, especially corticoids, and its fluorescent derivatives with anthroyl cyanide [5]. 6-Hydroxycorticoids, one of the most important unconjugated corticoids in human urine [5], were chosen as model compounds together with other familiar endogeneous and synthetic C_{21} steroids (FIGURE 1).

MATERIALS AND METHODS

Materials

CDs and corticoids were kindly supplied by Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan) and Teikoku Hormone Mfg. Co., Ltd. (Tokyo), respectively. 6-Hydroxycorticoids were synthesized from the respective corticoid according to the procedure described by Tsuji et al. [6] or Julian et al. [7]. 1- And 9-anthroyl cyanides were obtained from Wako Pure Chem. Ind., Ltd. (Osaka, Japan). The fluorescent derivatives were synthesized according to the procedure described by Goto et al. [5]. Solvents were purified by distillation prior to use.

Apparatus

HPLC was carried out on a JASCO TRI ROTAR chromatograph equipped with a JASCO UVIDEC-100-II ultraviolet detector (UV: 240 nm) (Japan Spectroscopic Co., Ltd., Tokyo) or Hitachi F-1000

fluorescence detector (FL: λ ex. 360 nm, λ em. 460 nm)(Hitachi Ltd., Tokyo). A TSKgel ODS-80TM (5 μm) column (15 cm x 0.46 cm i.d.)(TOSOH, Tokyo) was used at a flow rate of 1 ml/min.

RESULTS AND DISCUSSION

The Separation by Conventional Method

First we examined the separation of the pair of C_{21} steroids having the similar structure, such as the corticoids with or without 1-ene (**1** vs. **2**; **3** vs. **4**) and 6-hydroxy isomer (**5** vs. **6**; **7** vs. **8**; **9** vs. **10**; **11** vs. **12**), by conventional method using methanol or acetonitrile as an organic modifier. The results were summarized in TABLE 1 and the separation of cortisol (**1**) and prednisolone (**2**) has not been done by both solvent systems. On the contrary the satisfactory separation (Rs > 1.20) of all the other pairs of C_{21} steroids has been obtained by the solvent system using either methanol or acetonitrile as an organic modifier. All the 6 β-hydroxy compounds (**5, 7, 9, 11**) were eluted earlier than the respective 6 α-hydroxy isomer (**6, 8, 10, 12**) (TABLE 1).

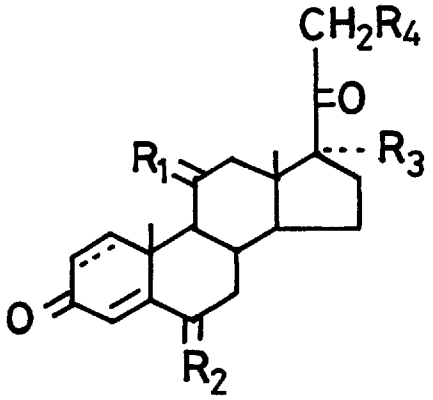
Recently Goto et al. reported the derivatization method using 1- or 9-anthroyl cyanide as a fluorescent labeling reagent and the application to the determination of corticoids in biological fluids [5]. According to these data, the separation of fluorescent derivatives with anthroyl cyanide was also examined by conventional method. The fluorescent derivative labeled at 21-

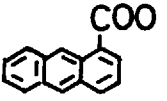
TABLE 1
Chromatographic Behavior of C_{21} Steroids *

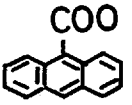
Pair of Steroids	Solvent System MeOH-H_2O	t_R (min)	Rs	Solvent System CH_3CN-H_2O	t_R (min)	Rs
1	(4:5)	24.7	0	(1:3)	15.2	0
2		24.4			15.2	
3	(4:5)	20.1	3.50	(1:3)	17.8	3.19
4		17.4			15.9	
5	(4:5)	6.4	1.80	(1:7)	13.9	1.18
6		7.2			14.7	
7	(4:5)	9.0	5.35	(1:5)	10.4	5.10
8		12.0			13.0	
9	(2:3)	11.4	2.22	(1:4)	11.7	2.39
10		13.1			13.6	
11	(3:2)	9.4	4.20	(1:1)	4.5	4.62
12		14.4			5.6	

* Detection: UV

position of steroid with 1- or 9-anthroyl cyanide was expressed as **a** or **b**, respectively (FIGURE 1), and results were summarized in TABLE 2. The satisfactory separation of 6-hydroxy-11-deoxycortisol isomer (**9b** vs. **10b**) and 5-position isomeric tetrahydrocortisol (**13b** vs. **14b**) derivatives each other has not been obtained by both solvent systems. All the other examined pairs of derivatives showed the Rs values of more than 1.20 with the solvent system using methanol or acetonitrile as an organic modifier.



a: R_4= 

b: R_4= 

1 : R_1=$\underset{H,}{\overset{\cdot\cdot\cdot}{\cdot}}$OH R_2=H_2, R_3=R_4=OH

2 : R_1=$\underset{H,}{\overset{\cdot\cdot\cdot}{\cdot}}$OH R_2=H_2, R_3=R_4=OH, Δ^1

3 : R_1=O, R_2=H_2, R_3=R_4=OH

4 : R_1=O, R_2=H_2, R_3=R_4=OH, Δ^1

5 : R_1=R_2=$\underset{H,}{\overset{\cdot\cdot\cdot}{\cdot}}$OH R_3=R_4=OH

6 : R_1=$\underset{H,}{\overset{\cdot\cdot\cdot}{\cdot}}$OH R_2=$\underset{OH,}{\overset{\cdot}{\cdot\cdot\cdot}}$H R_3=R_4=OH

7 : R_1=O, R_2=$\underset{H,}{\overset{\cdot\cdot\cdot}{\cdot}}$OH R_3=R_4=OH

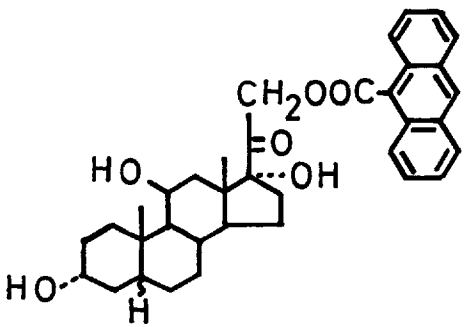
8 : R_1=O, R_2=$\underset{OH,}{\overset{\cdot}{\cdot\cdot\cdot}}$H R_3=R_4=OH

9 : R_1=H_2, R_2=$\underset{H,}{\overset{\cdot\cdot\cdot}{\cdot}}$OH, R_3=R_4=OH

10: R_1=H_2, R_2=$\underset{OH,}{\overset{\cdot}{\cdot\cdot\cdot}}$H R_3=R_4=OH

11: R_1=H_2, R_2=$\underset{H,}{\overset{\cdot\cdot\cdot}{\cdot}}$OH R_3=R_4=H

12: R_1=H_2, R_2=$\underset{OH,}{\overset{\cdot}{\cdot\cdot\cdot}}$H R_3=R_4=H



13b: 5β

14b: 5α

FIGURE 1. Structures of C_{21} steroids and its fluorescent derivatives

TABLE 2
Chromatographic Behavior of C_{21} Steroid Derivatives *

Pair of Derivatives	Solvent System MeOH-H_2O	t_R (min)	Rs	Solvent System CH_3CN-H_2O	t_R (min)	Rs
1a	(5:1)	10.0	1.41	(2:1)	13.2	1.66
2a		9.1			11.8	
3a	(5:1)	11.3	1.20	(2:1)	14.8	1.39
4a		10.4			13.4	
5b	(5:2)	9.6	1.40	(1:1)	15.3	0
6b		11.0			15.3	
7b	(5:2)	13.4	1.52	(1:1)	21.4	0
8b		15.4			21.4	
9b	(10:3)	13.8	0.57	(3:2)	15.8	1.07
10b		14.4			16.6	
13b	(3:1)	20.5	0.52	(8:5)	17.3	1.14
14b		21.3			18.3	

* Detection: FL

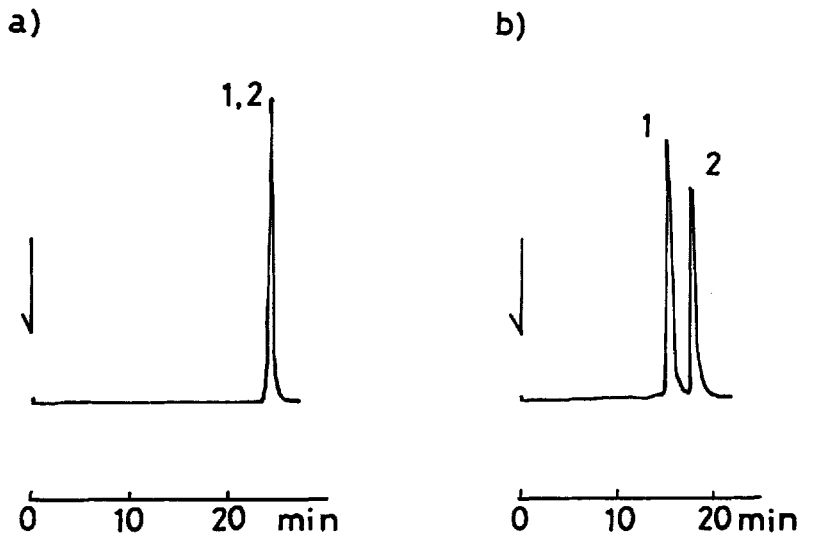


FIGURE 2. Separation of **1** and **2**
Conditions: detection, UV; mobile phase, a) MeOH-H_2O (4:5), b) MeOH-H_2O (4:5) containing 5 mM β-CD.

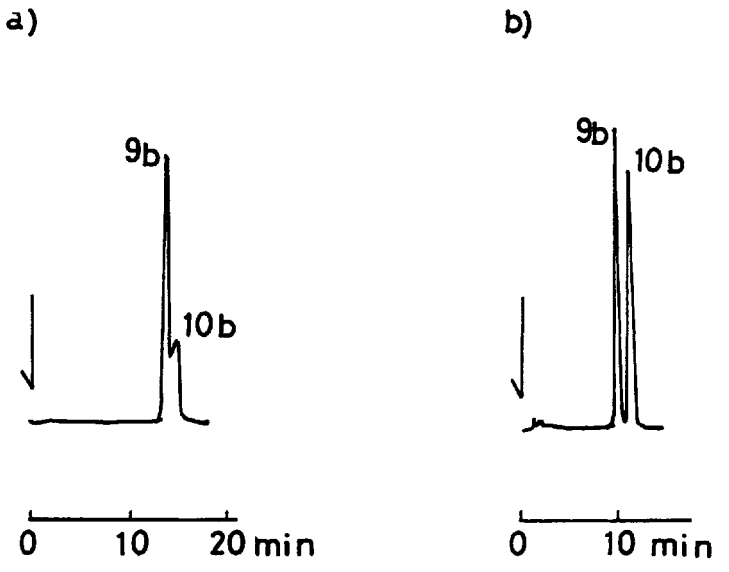


FIGURE 3. Separation of **9b** and **10b**
Conditions: detection, FL; mobile phase, a) MeOH-H_2O (10:3), b) MeOH-H_2O (10:3) containing 4 mM γ-CD.

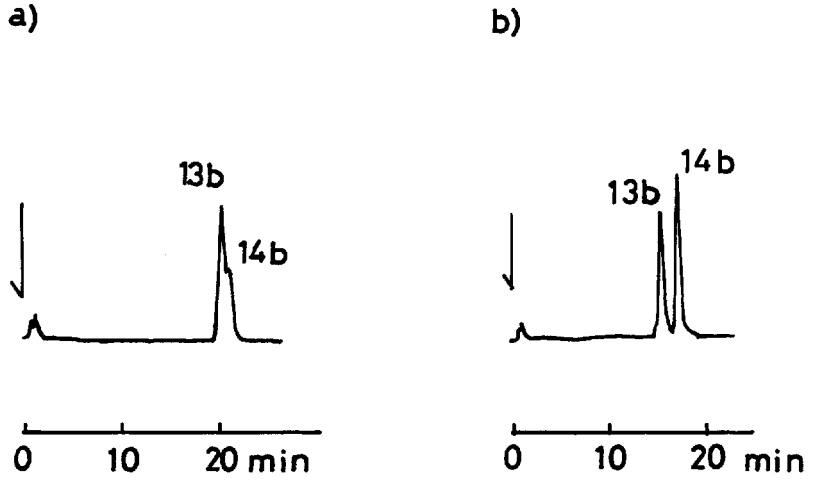


FIGURE 4. Separation of **13b** and **14b**
Conditions: detection, FL; mobile phase, a) MeOH-H_2O (3:1), b) MeOH-H_2O (3:1) containing 5 mM γ-CD.

Separation by Inclusion Chromatography

The inclusion chromatography using CD as a mobile phase additive was applied to the separation of three pairs of compounds (**1** vs. **2**; **9b** vs. **10b**; **13b** vs. **14b**) whose separation has not been done by the conventional method as described above. The addition of β- or γ-CD to the mobile phase much improved the separation of these compounds, whose chromatograms were shown in FIGURE 2-4.

These data showed the method is useful for the separation of C_{21} steroids and its fluorescent derivatives. Application of this method to the determination of corticoids in biological fluids are being conducted in these laboratories and the details will be reported elsewhere in the near future.

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