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Separation of Hydrocortisone and Epi-hydrocortisone by Solvent Extraction

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Abstract: Solvent extraction, instead of traditional crystallization, is suggested as a new method for separation of hydrocortisone and its optical isomer, epi-hydrocortisone. The extraction behavior of alcohols, ketones, esters, ethers and chlorinated hydrocarbons was studied experimentally. For the industrial separation process it was found that the best solvents are *n*-butyl acetate or chloroform. The distribution coefficients of hydrocortisone and epi-hydrocortisone in an *n*-butyl acetate/water system at 17°C were found to be 9·98 and 2·62, respectively, and 5·57 and 1·93, respectively, in a chloroform/water system at 17°C. When *n*-butyl acetate or chloroform solution of crude hydrocortisone (the mixture of hydrocortisone, epi-hydrocortisone and other steroid impurities) was scrubbed by deionized water in a nine-stage cross-current at 25°C, the organic phase hydrocortisone purity increased from 78·10% to 98·22% (wt%) for the *n*-butyl acetate case and from 78·10% to 98·02% (wt%) for the chloroform case. The medicinal standard for hydrocortisone was attained. The effects of alcohol concentration, temperature, salting-out and pH on extraction are also discussed.

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Key words: hydrocortisone, epi-hydrocortisone, optical isomer, extraction separation, *n*-butyl acetate, chloroform

NOTATION

- C Concentration (mg dm^{-3})
- D Distribution coefficient, ratio of organic concentration to aqueous concentration
- f Frequency (cm^{-1})
- N Number of stages in counter-current scrubbing
- P Purity of hydrocortisone, weight percentage of hydrocortisone in solutes, in organic phase (wt%)
- S_c Separation factor, D_β/D_α
- S_{in} Solubility of solvent in water (g solvent in 100 g water)
- S_m Solubility of hydrocortisone in solvent (g water in 100 g solvent)
- t_b Boiling point of solvent (°C)
- T Transmittance (%)

Subscripts

- o Organic phase
- w Aqueous phase
- α Epi-hydrocortisone
- β Hydrocortisone

1 INTRODUCTION

Many steroids in nature are optically active. The production and separation of optically active compounds are increasing rapidly in the pharmaceutical industry.[1–3] Hydrocortisone, known as cortisol or Kendall's compound F, is an important steroid drug. Epi-hydrocortisone is one of the optical isomers of hydrocortisone. The specific rotatory power of hydrocortisone is $[\alpha]_D^{22} = +167°$ (in ethyl alcohol) while that of epi-hydrocortisone is $[\alpha]_D^{22} = +117°$ (in ethyl alcohol).[4] There is little difference in the space orientation of their C_{11} hydroxyl groups. Hydrocortisone has

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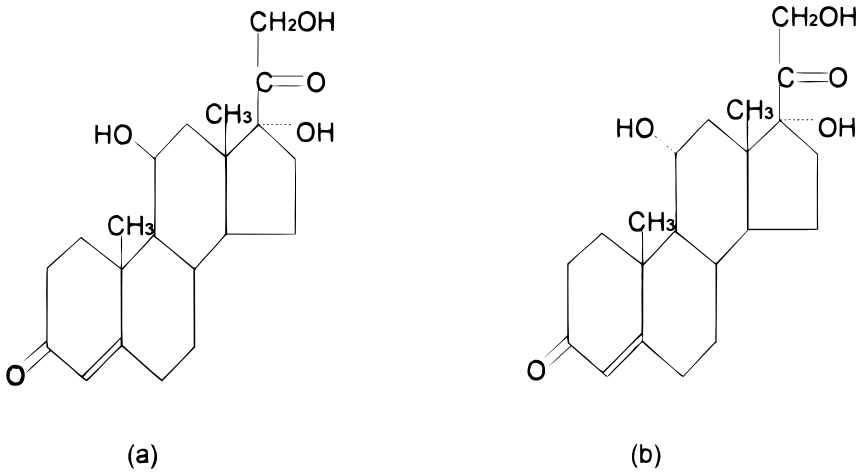

Fig. 1. Nomenclature and structure of hydrocortisone(a) and epi-hydrocortisone(b): (a) hydrocortisone, 11β,17α,21-trihydroxy-pregn-4-ene-3,20-dione; (b) epi-hydrocortisone, 11α,17α,21-trihydroxy-pregn-4-ene-3,20-dione.

an 11β substituent but epi-hydrocortisone has an 11α substituent, as shown in Fig. 1. (the solid line represents β substituents and the dashed line represents α substituents). The difference in their steric structures causes a slight difference in their physical and chemical properties.

Hydrocortisone has been used to treat the symptoms of many diseases but epi-hydrocortisone is less pharmacologically active. Due to the low selectivity of the oxidizing reaction in the hydrocortisone manufacturing process, epi-hydrocortisone is always produced as a side-product of the hydrocortisone. The crude product must be purified to get pure hydrocortisone.[5]

The common methods used for separating hydrocortisone and epi-hydrocortisone are chromatography[6–8] and crystallization,[5,9–11] due to the different retention times for hydrocortisone and epi-hydrocortisone in some chromatography systems and different solubilities in some solvents. The former is often used for purification or preparation in the laboratory, while the latter is more commonly used in manufacturing. High energy consumption and complex operations have always been serious problems in hydrocortisone manufacturing, consequently, the crystallization process needs improvement. Solvent extraction with its low energy consumption is easy and attractive.

In this work, it was found that hydrocortisone and epi-hydrocortisone have different distribution coefficients in some organic solvent/aqueous solution systems. Solvent extraction was tested to separate hydrocortisone and epi-hydrocortisone.

2 EXPERIMENTAL

2.1 Materials and reagents

Medicinal hydrocortisone, medicinal epi-hydrocortisone and crude hydrocortisone were all supplied by a factory. The hydrocortisone content in medicinal hydrocortisone is above 98% (wt%) and the epi-hydrocortisone content in medicinal epi-hydrocortisone is above 97% (wt%). In addition to hydrocortisone and epi-hydrocortisone, crude hydrocortisone also contains other steroid impurities.[12] The content of these impurities is below 8% (wt%) while hydrocortisone and epi-hydrocortisone are in a ratio of 3·5–4 : 1(wt/wt).

All other reagents used were analytical purity grade.

2.2 Preparation of organic solution and aqueous solution

A specified amount of hydrocortisone, epi-hydrocortisone, or crude hydrocortisone was dissolved in *n*-butyl acetate or chloroform at 50°C, and the *n*-butyl acetate or chloroform solution of hydrocortisone, epi-hydrocortisone or crude hydrocortisone was obtained.

The hydrocortisone, or epi-hydrocortisone was added to deionized water, heated for 20–30 min at 80°C, and then cooled and filtered to get a saturated aqueous solution at room temperature. Because hydrocortisone solubility in water is low (0·028 g per 100 g water, 25°C),[6] it is difficult to prepare highly concentrated aqueous solutions. Hydrocortisone or epi-hydrocortisone could be dissolved in alcohol and then diluted with deionized water, producing a 5% alcohol–water solution of hydrocortisone (or epi-hydrocortisone).

2.3 Experiments of phase equilibrium and analytical methods

Two phases were mixed in an Erlenmeyer flask with a pre-determined volume ratio. Then the mixture was stirred while immersed in a water bath at a pre-determined temperature for 5–10 min. Finally, the two

phases were separated by centrifugation. Samples were taken from each phase, evaporated and dried at 80°C, and then dissolved in ethyl alcohol and analyzed.

The concentration of hydrocortisone or epi-hydrocortisone in the two phases was analyzed by ultra-violet spectrophotometry at a wavelength of 241 nm. The crude hydrocortisone composition was analyzed by high performance liquid chromatography, using a Gilson-715 HPLC system. The column used was a Zorbax C8 reverse-phase chromatographic column, 4 mm in i.d. and 15 cm in length, with a mobile phase of 70% methanol–water solution (vol.%) and a flow rate of 1·0 cm^3 min^{-1}. An ultra-violet detector was used.

3 RESULTS AND DISCUSSION

3.1 Solvent selection

Phase equilibrium experiments were used to determine distribution coefficients of hydrocortisone and epi-hydrocortisone in some organic solvent/saturated aqueous solution systems. The results are listed in Table 1.

The distribution coefficient of hydrocortisone or epi-hydrocortisone was largest in alcohols and decreased in the following order based on the same or similar number of carbon atoms: ketones > esters > chlorinated hydrocarbons > ethers > hydrocarbons. The distribution coefficients of hydrocortisone and epi-hydrocortisone were smallest in aromatic hydrocarbons and cyclohexane. Moreover, the distribution coefficient of hydrocortisone was larger than that of epi-hydrocortisone in these solvent systems.

As shown in Fig. 1, although hydrocortisone or epi-hydrocortisone has a non-polar steroid nucleus, the polar substituents, hydroxyl (C11, C17 and C21) and carbonyl (C3 and C20), increase its polarity to some extent. These polar groups may form a hydrogen bond with chloroform, dichloromethane (known as class A solvents), ketones, esters, ethers (class B solvents), or alcohols (class AB solvents). However, they cannot form a hydrogen bond with an inert solvent such as tetrachloromethane, benzene or cyclohexane. Since hydrocortisone or epi-hydrocortisone can form their strongest hydrogen bond with alcohols, alcohols have the largest distribution coefficient for hydrocortisone and epi-hydrocortisone. Moreover, the strength of the hydrogen bond between the solute and solvent decreases in the following order: ketones > esters > ethers.[13] Therefore, the extracting powers of these solvents should decrease in the same order. Chloroform and dichloromethane are well known hydrogen bond donors, but interaction between them and hydrocortisone or epi-hydrocortisone is not strong enough to make their distribution coefficients as large as those of alcohols, ketones or esters.

Hydrogen bonding of hydrocortisone with some solvents was confirmed by an infrared spectrogram. Infrared absorption spectra of the hydroxyl group of hydrocortisone in saturated acetone, 2-pentanone, ethyl acetate and *n*-butyl acetate solution (solvent absorption was eliminated) are shown in Fig. 2. Generally, the characteristic vibration frequency of a free hydroxyl group is about 3600–3650 cm^{-1}. When a hydroxyl

TABLE 1
Distribution Coefficients of Hydrocortisone and Epi-hydrocortisone and Separation Factors in Organic Solvent/Saturated Aqueous Solution Systems, 17°C

Alcohols				*Ketones*				*Esters*			
Solvent	D_β	D_α	S_c	*Solvent*	D_β	D_α	S_c	*Solvent*	D_β	D_α	S_c
1-Pentanol	34·80	31·20	1·12	2-Pentanone	27·10	9·02	3·00	Ethyl acetate	11·62	3·40	3·42
Iso-pentanol	30·70	26·69	1·15	2-Hexanone	26·12	8·50	3·07	Propyl acetate	10·38	2·89	3·59
1-Hexanol	28·62	18·27	1·57	MIBK[a]	14·17	5·10	2·78	Isopropyl acetate	11·50	3·28	3·50
1-Octanol	14·07	8·14	1·73	2-Heptanone	14·92	4·84	3·08	*n*-Butyl acetate	9·98	2·62	3·80
2-Ethyl-hexanol	14·86	9·20	1·61								

Ethers				*Chlorinated hydrocarbons*				*Aromatic hydrocarbons and cyclohexane*			
Solvent	D_β	D_α	S_c	*Solvent*	D_β	D_α	S_c	*Solvent*	D_β	D_α	S_c
Ethyl ether	1·60	0·42	3·80	Dichloromethane	5·18	1·78	2·90	Benzene	0·37	0·04	9·25
Propyl ether	0·85	0·33	2·58	Chloroform	5·57	1·93	2·89	Toluene	0·19	0·02	9·50
Butyl ether	0·18	0·09	2·00	Tetrachloromethane	0·39	0·20	1·95	Xylene	0·11	0·01	11·0
				1,2-Dichloroethane	1·90	0·95	2·00	Cyclohexane	0·07	0·01	7·00
				1,1,1-Trichloroethane	0·25	0·16	1·56				

[a] MIBK: Methyl isobutyl ketone.

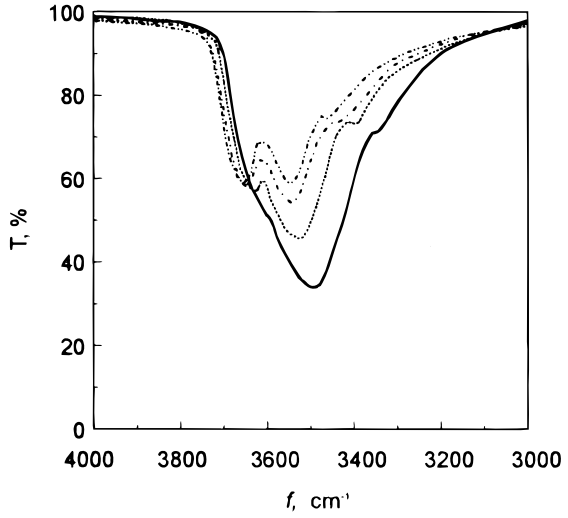

Fig. 2. Infrared absorption spectra of hydrocortisone in different organic solvents. ——, in acetone; ···, in 2-pentanone; – ·–·, in ethyl acetate; –···–, in *n*-butyl acetate.

group of a solute has a greater hydrogen bonding strength, the characteristic frequency of the hydroxyl group may decrease.[14] Hydrocortisone spectra in acetone showed a single absorption peak with a frequency of 3498·0 cm^{-1}. This means that acetone had strong hydrogen bonding with all of hydrocortisone hydroxyl groups. In 2-pentanone, ethyl acetate or *n*-butyl acetate, two obvious absorption peaks appeared. The frequencies of the first absorption peaks were 3625·8 cm^{-1}, 3630·1 cm^{-1} and 3640·0 cm^{-1}. This means that a part of the hydroxyl group was free and was having difficulty in forming a hydrogen bond with the solvent. The frequencies of the second absorption peaks were 3521·3 cm^{-1}, 3539·3 cm^{-1} and 3540·4 cm^{-1}. This part of the hydroxyl had hydrogen bonding interaction with the solvent. The power to form a hydrogen bond decreased in the order: 2-pentanone > ethyl acetate > *n*-butyl acetate. The frequency of the second absorption peaks and the area of free hydroxyls increased in the same order.

The difference in space structure of hydrocortisone and epi-hydrocortisone may be responsible for the difference in their distribution coefficients. According to sterochemistry, the 11β hydroxyl of hydrocortisone is an axial substituent while the 11α hydroxyl of epi-hydrocortisone is an equatorial substituent. In some reactions the latter is more stable than the former.[15] It is reasonable to consider that there is larger steric hindrance because the equatorial substituent is affected remarkably by the 'plane' of the skeleton. Therefore the interaction between the 11α hydroxyl of epi-hydrocortisone and a solvent molecule may have been weakened by steric hindrance. Thus, the distribution coefficient of epi-hydrocortisone was smaller than that of hydrocortisone in the solvent/water systems studied. In other words, the separation factor of these solvents, S_c, was always larger than 1. The largest separation factor was that of aromatic hydrocarbons (and cyclohexane), then it decreased in the following order: ethyl ether > esters > ketones > chloroform (and dichloromethane). The smallest separation factor was that of the alcohols.

As an extractant for separating epi-hydrocortisone from hydrocortisone, the solvent should have not only a larger distribution coefficient (D_β) and a larger separation factor, but also lower solubility in water and a moderate boiling point for easy regeneration of solvent. It was found experimentally that *n*-butyl acetate and chloroform could be used for the industrial process. The properties of these candidates are shown in Table 2.

3.2 Determination of distribution curve

The *n*-butyl acetate (or chloroform) solutions of hydrocortisone and epi-hydrocortisone were equilibrated with deionized water at various phase ratios (volume ratio) to determine their distribution curves. The results are shown in Fig. 3. For low concentrations (0–300 mg dm^{-3}) the distribution coefficient of hydrocortisone was about 9·98 and that of epi-hydrocortisone was 2·62 in *n*-butyl acetate/aqueous solution systems at 17°C. In a chloroform/aqueous solution system, the corresponding distribution coefficients were 5·57 and 1·93 at 17°C. In the concentration range of 0–300 mg dm^{-3} the separation factors of these two solvents remained constant. The separation factor in *n*-butyl acetate at 17°c was about 3·80, while that in chloroform was 2·89 at the same temperature.

3.3 Factors effecting distribution

3.3.1 Ethyl alcohol concentration

The fermentation broth in hydrocortisone production always contains 4–5% (vol.%) ethyl alcohol.[5] This may affect the distribution behavior of hydrocortisone or epi-hydrocortisone. Distribution coefficients were determined in *n*-butyl acetate/5% ethyl alcohol(vol.%) aqueous solution and chloroform/5% ethyl alcohol aqueous solution systems at 17°C. In aqueous phases

TABLE 2
Properties of Extractants

	n-*Butyl acetate*	*Chloroform*
D_β (15°C)	9·98	5·57
S_c [a]	3·80	2·89
S_{in},[16] g per 100 g water (20°C)	1·0	0·8
t_b,[16] °C	126·2	63·5
S_m,[b] g dm^{-3} (25°C)	2·91	2·10

[a] Quoted from Table 1.
[b] The solubility of hydrocortisone in *n*-butyl acetate and chloroform at 25°C determined in this work.

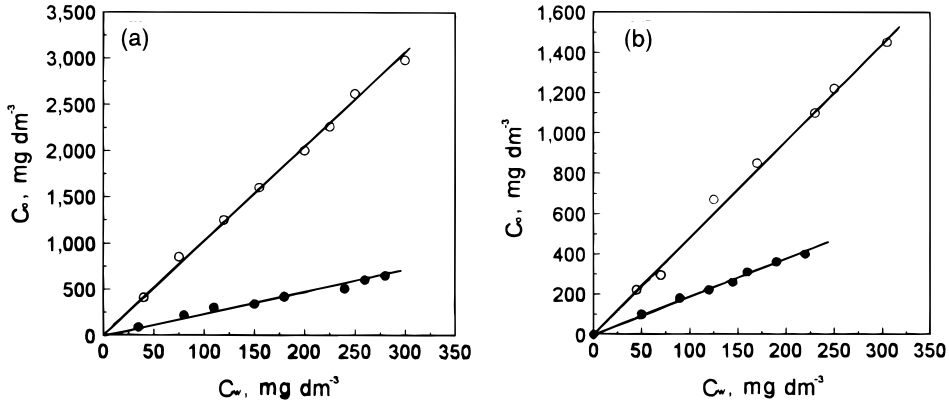

Fig. 3. Distribution of hydrocortisone and epi-hydrocortisone in *n*-butyl acetate/water system(a) and chloroform/water system(b), 17°C. ○, Hydrocortisone; ●, epi-hydrocortisone.

with a range of 0–300 mg dm^{-3}, when aqueous concentration increased, the organic concentration of hydrocortisone or epi-hydrocortisone increased in proportion to the aqueous concentration. The distributions of hydrocortisone and epi-hydrocortisone were different due to the different distribution behavior of ethyl alcohol in *n*-butyl acetate and chloroform. The distribution coefficients of hydrocortisone and epi-hydrocortisone in *n*-butyl acetate/5% ethyl alcohol–water were 9·89 and 2·58, respectively and were slightly smaller than those in an *n*-butyl acetate/water system (9·98 and 2·62). However, the distribution coefficients of hydrocortisone and epi-hydrocortisone in a chloroform/5% ethyl alcohol–water system were 5·65 and 2·00, respectively, slightly larger than those in a chloroform/water system (5·57 and 1·93). In this work, the influence of 5% ethyl alcohol added in the aqueous phase was ignored. When the concentration of ethyl alcohol in the aqueous phase increased, it seriously affected the distribution of hydrocortisone.[6]

3.3.2 *Temperature effect*

At temperatures of 15°C, 25°C and 35°C, the distribution coefficients in the *n*-butyl acetate system and chloroform system were determined. The results are shown in Fig. 4.

In the *n*-butyl acetate system, the temperature effect on the hydrocortisone distribution was not obvious. The distribution coefficients for hydrocortisone were about 9·90, 10·10 and 10·35 at 15°C, 25°C and 35°C respectively. The distribution coefficient of epi-hydrocortisone increased from 2·55 to 3·10 as the temperature increased from 15°C to 35°C.

In the chloroform system, the distribution coefficients of hydrocortisone and epi-hydrocortisone increased as temperature increased. The distribution coefficient of hydrocortisone increased from 4·70 to 7·80 and that of epi-hydrocortisone increased from 1·65 to 2·30 when temperature increased from 15°C to 35°C.

Because the distributions of hydrocortisone and epi-hydrocortisone were affected differently by temperature in *n*-butyl acetate and chloroform systems, the separation factor of *n*-butyl acetate decreased from 3·88 to 3·34 and that of chloroform increased from 2·85 to 3·39 when the temperature increased from 15°C to 35°C.

3.3.3 *Salting out effect*

Ammonium sulfate was added in the aqueous phase to observe the salting out effect on the distribution. Figure

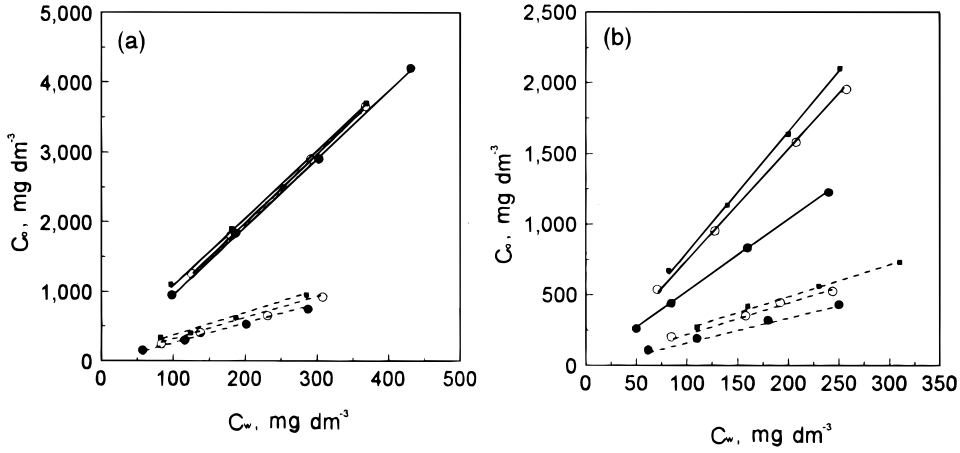

Fig. 4. Effect of temperature on distribution of hydrocortisone and epi-hydrocortisone in *n*-butyl acetate system(a) and chloroform system(b). ●, 15°C; ○, 25°C; ■, 35°C. ——, Hydrocortisone, – – –, epi-hydrocortisone.

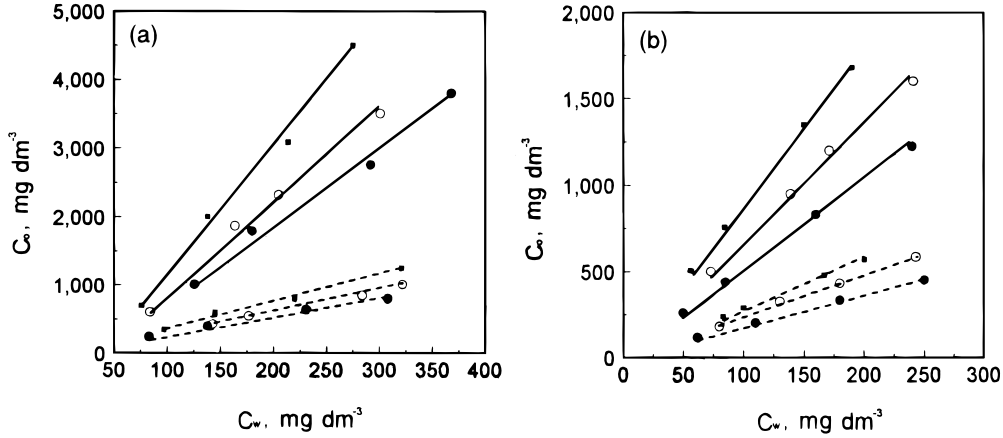

Fig. 5. Salting out (ammonium sulfate) effect on distribution of hydrocortisone and epi-hydrocortisone in *n*-butyl acetate system(a) and chloroform system(b), 25°C. ●, 0·0% (wt%) $(NH_4)_2SO_4$; ○, 0·8% (wt%) $(NH_4)_2SO_4$; ■, 2·5% (wt%) $(NH_4)_2SO_4$. ——, Hydrocortisone; – – –, epi-hydrocortisone.

5 shows the distribution coefficients of hydrocortisone and epi-hydrocortisone in *n*-butyl acetate/ammonium sulfate in 5% ethyl alcohol aqueous solution or chloroform/ammonium sulfate in 5% ethyl alcohol aqueous solution systems at 25°C. The aqueous concentrations of ammonium sulfate were 0·0%, 0·8% and 2·5% (wt%), respectively.

Since the added inert electrolyte affected the activity coefficient of the solute in aqueous solution, the distribution coefficients of both hydrocortisone and epi-hydrocortisone increased with increasing salt concentration. The distribution coefficients of hydrocortisone and epi-hydrocortisone increased respectively from 10·10 to 13·50 and from 2·95 to 3·80 in the *n*-butyl acetate system, while in the chloroform system, the distribution coefficients changed from 6·95 to 9·52 and from 2·00 to 2·90. However, the salting out effect on the separation factor was not remarkable.

3.3.4 *pH effect*

The effect of the hydrogen ion concentration on distribution in the aqueous phase was determined at pH 3·9, 7·0 and 10·9 (25°C). The results are shown in Fig. 6.

The distribution coefficients of both hydrocortisone and epi-hydrocortisone decreased in the acidic and alkaline systems. The distribution coefficient of hydrocortisone decreased from 10·10 to 9·57 in the acidic condition, but decreased from 10·1 to 6·82 in the alkaline condition for the *n*-butyl acetate system. The distribution coefficient of epi-hydrocortisone decreased from 2·95 to 2·25 at acid pH and from 2·95 to 1·99 in an alkaline pH. Both distribution coefficients had their largest values at pH 7.

As shown in Fig. 6(b), in the chloroform system, the pH influence on the hydrocortisone distribution behavior was small in acidic conditions but larger in alkaline conditions, similar to that for the *n*-butyl acetate system.

As the pH increased from 3·9 to 10·9, the separation factor decreased from 4·25 to 3·40 in the *n*-butyl acetate system, but from 4·45 to 3·05 in the chloroform system.

Generally, the steroid nuclei of hydrocortisone and epi-hydrocortisone are non-polar and ionization of substituents is weak. However, it is known that hydrocortisone and epi-hydrocortisone will be oxidized in strong acidic or basic solutions, resulting in rearrangement or degradation.[6] The decrease in distribution coefficients

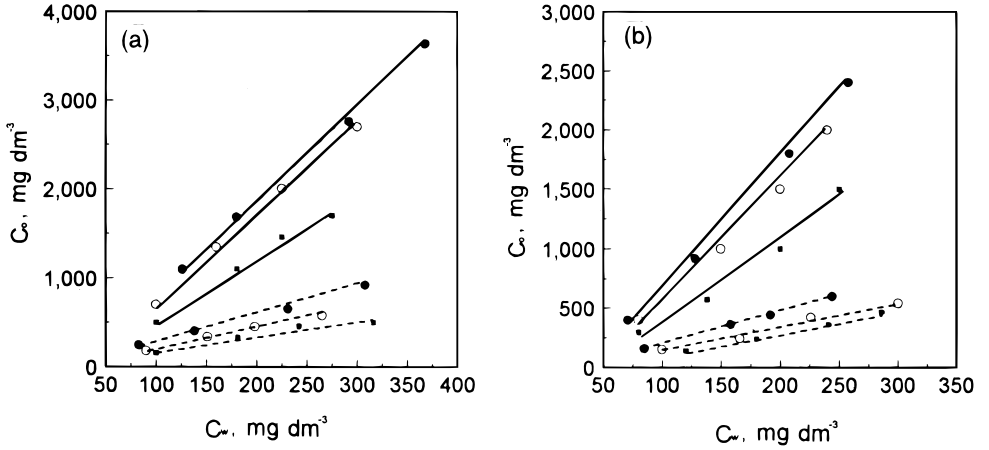

Fig. 6. Effect of pH on distribution of hydrocortisone and epi-hydrocortisone in *n*-butyl acetate system(a) and chloroform system(b), 25°C. ●, pH = 7·0; ○, pH = 3·9; ■, pH = 10·9. ——, Hydrocortisone; – – –, epi-hydrocortisone.

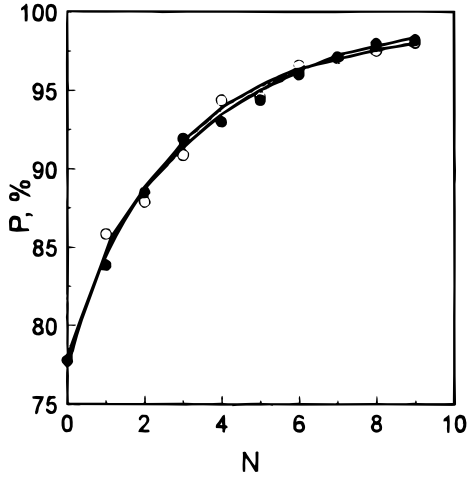

Fig. 7. Separation of hydrocortisone and epi-hydrocortisone by scrubbing from loaded *n*-butyl acetate and chloroform solution with deionized water, 25°C. ●, *n*-Butyl acetate; ○, chloroform. (*N* is number of stages in counter-current scrubbing. *P* is purity of hydrocortisone, weight percentage of hydrocortisone in solutes, in organic phase, wt%).

may be on account of this situation. The mechanism of pH effect on distribution behavior will be investigated in the future.

3.4 Results of solvent extraction

The purity of crude hydrocortisone can be increased from 75–80% (wt%) to 98% (wt%) by crystallization in industrial production. To simplify experimental operation, the cross-current stages were used for checking the effectiveness of solvent extraction.

The *n*-butyl acetate and chloroform solutions of crude hydrocortisone were scrubbed with deionized water in a nine-stage cross-current at the phase ratio of $A/O = 2{\cdot}5:1$ (volume ratio). Hydrocortisone purity (weight percentage of hydrocortisone in solutes) in the organic phase varied with the cross-current stage number, as shown in Fig. 7.

As the distribution coefficient of epi-hydrocortisone between two phases was smaller, it was removed gradually from the organic phase with increase of the scrubbed stage number. It was found that the hydrocortisone purity in the organic phase increased from 78·10% to 98·22% in an *n*-butyl acetate system while it increased from 78·10% to 98·02% in a chloroform system after nine-stage scrubbing. Hydrocortisone of medicinal standard was attained.

The counter-current stages, of course, will be used in commercial production to save solvent.

4 CONCLUSIONS

1. Separation of hydrocortisone and epi-hydrocortisone by solvent extraction is an attempt to separate optically active compounds. Compared with other separation methods such as unsymmetrical synthesis, crystallization, chromatography and the enzyme action method, solvent extraction has the following advantages: lower cost, lower energy consumption and easier operation. It can be expected that this new technique will be developed and used in commercial production.

2. With moderate boiling points and low solubility in water, *n*-butyl acetate and chloroform were chosen as extractant to separate hydrocortisone and epi-hydrocortisone. Experimental results confirmed that hydrocortisone purity obtained by solvent extraction attained the standard of medicinal hydrocortisone.

3. Temperature, salting out and pH effects on distribution in an *n*-butyl acetate system were similar to those in a chloroform system. The distribution coefficients of hydrocortisone and epi-hydrocortisone decreased in an *n*-butyl acetate system while they increased in a chloroform system when ethyl alcohol was added in the aqueous phase.

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