[Chem. Pharm. Bull. 33(11)5113—5118(1985)]

Percutaneous Absorption and Metabolism of Hydrocortisone Butyrate Propionate in Dog Skin

YASUO OZAWA,*,[a] IKUO KOYAMA,[a] SUSUMU MURAYAMA[a]
and TANEKAZU NADAI[b]

*Research Center, Taisho Pharmaceutical Co., Ltd.,[a] 1-403, Yoshino-cho,
Ohmiya, Saitama 330, Japan and Faculty of Pharmaceutical Science,
Setsunan University,[b] Nagaotogecho, Hirakata,
Osaka 573-01, Japan*

(Received March 11, 1985)

Percutaneous absorption and skin metabolism of ^{3}H-hydrocortisone butyrate propionate, as a diester derivative of hydrocortisone, were studied *in vivo*. The agent was dissolved in saline and applied to the shaved abdominal skin of dogs.

Most of the absorbed hydrocortisone butyrate propionate was present in an unmetabolized form in the stratum corneum. In the stratum corneum-removed skin, 80% of the corticosteroid in rats and about 50% in dogs were found as metabolites. Therefore, the esterase activity in this layer is stronger than in the stratum corneum, and rats have stronger esterase activity than dogs. It appears that skin metabolism significantly affects the biological activity of esterified corticosteroid.

Keywords——hydrocortisone butyrate propionate; corticosteroid; skin; absorption; metabolism

Corticosteroids have been frequently used in topical dosage forms for cutaneous diseases. In recent years, separation of the drug effect from side effects has been an important area of investigation. Many studies have been made of the effects of esterification at the 17 and 21 positions.

McKenzie and Atkinson[1)] assayed the topical potency of betamethasone and 23 of its esters, using a vasoconstriction assay. It was found that ester derivatives are more active than the parent compound. Betamethasone dipropionate and hydrocortisone butyrate propionate have been more useful than betamethasone valerate and hydrocortisone butyrate, respectively, in the clinical management of psoriasis,[2,3)] because an increase in molecular lipophilicity improves percutaneous absorption.

Skin contains many kinds of enzyme and metabolic activities.[4)] For esterified steroids to be useful, they should be structurally modified, because metabolism in the skin is an important factor in percutaneous absorption. The relationship between the chemical structure of steroids and the affinity for the glucocorticoid receptor as a measure of drug effect has been investigated in recent years,[5)] and a kinetic study of esterified steroid in skin would have fundamental significance.

In previous studies, the authors examined the metabolism of hydrocortisone derivatives in the rat skin *in vitro*[6)] and *in vivo*.[7)] Täuber and Toda examined the metabolism of diflucortolone valerate and reported that the rate of hydrolysis in man was considerably lower than that in rats.[8)] In the present study, we investigated the dog skin metabolism of hydrocortisone butyrate propionate, one of the steroid-17,21-diesters, for which metabolizing enzyme activity is weak.

Experimental

Materials——[1,2-^{3}H]Hydrocortisone butyrate propionate was synthesized at our institute as follows.[9)] [1,2-

^{3}H]Hydrocortisone was suspended in anhydrous benzene, then methylorthobutyrate and p-toluenesulfonic acid were added, and the mixture was refluxed, yielding [1,2-^{3}H]cortisol-17,21-cyclomethylorthobutyrate. This was dissolved in methanol and refluxed with acetate buffer to obtain [1,2-^{3}H]hydrocortisone butyrate, which was esterified with pyridine–propionic anhydride to give [1,2-^{3}H]hydrocortisone butyrate propionate. The purity of the labeled compound was examined on silica gel thin-layer chromatography (TLC) developed with n-hexane–acetone–chloroform (4 : 3 : 3). Specific radioactivity was 296 μCi/mg, and the radiochemical purity was not less than 99%.

All reagents were of analytical grade. The chromatographic solvents were of high performance liquid chromatography (HPLC) grade.

***In Vivo* Absorption Study**——Female dogs, weighing about 10 kg, were anesthetized with urethane. Abdominal hair was removed with electric hair clippers. Cylindrical glass cells of 2.0 cm^2 area were fixed on the skin, and 2 μg of [1,2-^{3}H]hydrocortisone butyrate propionate dissolved in 0.5 ml of saline was put into each cell. In order to prevent evaporation of the sample solution, the cell was covered with a Teflon membrane.

All samples were recovered 0.5, 1, 2, 3 and 5 h after administration. The site of administration was then washed with approximately 3 ml of saline 3 times, and the washings were combined with the samples to bring the entire volume up to 10 ml. Part of the combined solution was mixed with a scintillator, and the radioactivity was determined on a liquid scintillation counter to obtain the residual ratio of [1,2-^{3}H]hydrocortisone butyrate propionate with respect to the administered dose.

Determination of Retention in the Skin and Intradermal Distribution——The residual sample in the cell on the skin was recovered 5 h after the percutaneous administration. Then, the stratum corneum at the site of administration was removed by stripping 20 times with cellophane adhesive tape. The compound in the stratum corneum on the tape was extracted with 15 ml of methanol 3 times with a total volume of 50 ml after filtration.

Next, the epidermis, except the stratum corneum and corium, was excised. This fraction (stratum corneum-removed skin) was extracted with 15 ml of ethyl acetate 3 times, for a total volume of 50 ml. A part of both fractions was evaporated to dryness, and the radioactivity was determined in a liquid scintillation counter.

Determination of Metabolites in the Skin——Isolation and assay of the unchanged steroid and its metabolites in the stratum corneum and of the stratum corneum-removed skin were performed by means of HPLC, with the extract prepared as described above. The extract was evaporated to dryness, and the dry residue was redissolved in the HPLC mobile phase. Non-labeled compounds were added as the carrier, and the solution was injected into the HPLC. After collecting the peak fractions corresponding to hydrocortisone butyrate propionate, hydrocortisone butyrate, hydrocortisone and others, the solvents in each fraction were evaporated off, and the ratio of the metabolites in each fraction in the skin to the total radioactivity was obtained by measurement with a liquid scintillation counter. Results of *in vivo* experiments were expressed as the mean ± S.D. of 3 experiments.

Preparation of the Crude Enzyme Extract and Enzymatic Reaction *in Vitro*——Male Wistar rats, weighing about 200 g, and female dogs, weighing about 10 kg, were used in the present study. Human plasma was separated from the blood of three male volunteers. The procedure for preparing the crude enzyme extracts was the same as that reported previously.[6)] Briefly, 1 g of skin or liver was excised from an animal, 18 ml of 1.15% (w/v) KCl solution was added, and the whole was homogenized under ice cooling. The homogenate was centrifuged at 0 °C and 10000 × ***g*** for 30 min. The supernatant thus obtained was used as the crude enzyme extract. The protein concentration in the crude enzyme extract was determined by the method of Lowry *et al.*[10)] Plasma was diluted with 18 ml of 1.15% (w/v) KCl solution.

Substrate solution (18 ml) in which 35.1 g of hydrocortisone butyrate propionate had been dissolved in 18 ml of 1/30 M phosphate buffer, pH 7.4, was placed in a 50 ml glass-stoppered flask. The flask was kept at 37 ± 0.5 °C for 5 min, then 0.2 ml of the crude enzyme extract was added. In the case of plasma, 0.2 ml of plasma, diluted with 18 ml of 1.15% (w/v) KCl solution as described above, was added to the substrate solution. Aliquots (2 ml) of the reaction solution were withdrawn at appropriate time intervals for analysis by HPLC. The reaction solution was extracted with 10 ml of ethyl acetate, and 8 ml of the organic phase was evaporated to dryness. The residue was redissolved in the HPLC mobile phase. Results of *in vitro* experiments were expressed as the mean ± S.D. of 3 experiments.

Instrumentation——A chromatograph equipped with a 254 nm ultraviolet (UV) monitor was used with a 150 × 4 mm i.d. column, containing Nucleosil C-18. The column temperature was set at 60 °C. A mixture of methanol, water, and acetic acid (54.5 : 45 : 0.5) was used as a mobile phase at a flow rate of 1 ml/min.

Results

A saline solution of hydrocortisone butyrate propionate was administered to the skin of a dog, after removal of the hair. The residual ratio of hydrocortisone butyrate propionate decreased gradually, as shown in Fig. 1; 83.6% of the drug remained unabsorbed 5 h after administration.

The sample solution remaining on the skin 5 h after the percutaneous administration was recovered. The skin was fractionated into stratum corneum and stratum corneum-removed

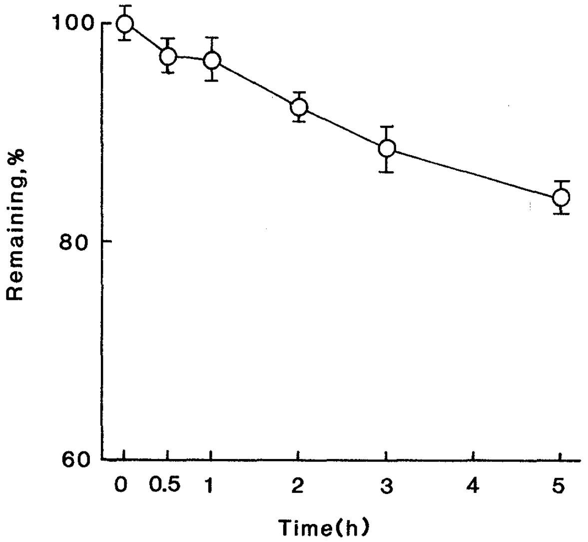

Fig. 1. Plot of Hydrocortisone Butyrate Propionate Remaining in Saline Solution against Time in Dogs

Initial concentration was 4 μg/ml.

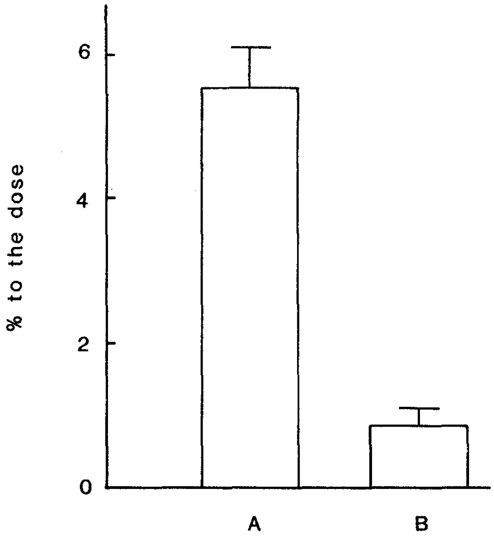

Fig. 2. Distribution of Radioactivity in Stratum Corneum Fraction (A) and Stratum Corneum-Removed Fraction (B) 5 h after Percutaneous Absorption

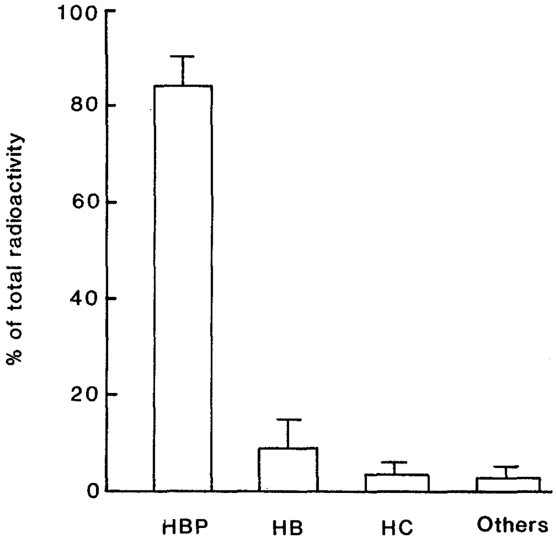

Fig. 3. Ratio of Unchanged Compound to Metabolites in the Stratum Corneum Fraction at 5 h after Administration

Hydrocortisone butyrate propionate (HBP), hydrocortisone butyrate (HB), hydrocortisone (HC), unknown metabolite (others).

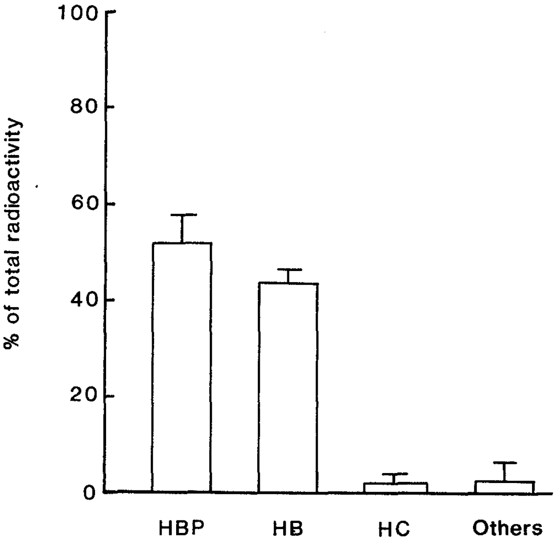

Fig. 4. Ratio of Unchanged Compound to Metabolites in the Stratum Corneum-Removed Fraction at 5 h after Administration

Hydrocortisone butyrate propionate (HBP), hydrocortisone butyrate (HB), hydrocortisone (HC), unknown metabolite (others).

skin, and the ratio of radioactivity in each fraction was obtained. As shown in Fig. 2, the accumulation of radioactivity in the skin was 5.6% in the stratum corneum fraction and 0.9% in the stratum corneum-removed fraction.

The ratios of unchanged hydrocortisone butyrate propionate and its metabolites in the skin are shown in Figs. 3 and 4. The authors$^{6)}$ have already found that the acyl group in the 21-position of hydrocortisone butyrate propionate is predominantly hydrolyzed; thereafter, the substituent of the 17-position is transferred nonenzymatically to the 21-position, and then the compound is finally hydrolyzed to hydrocortisone.

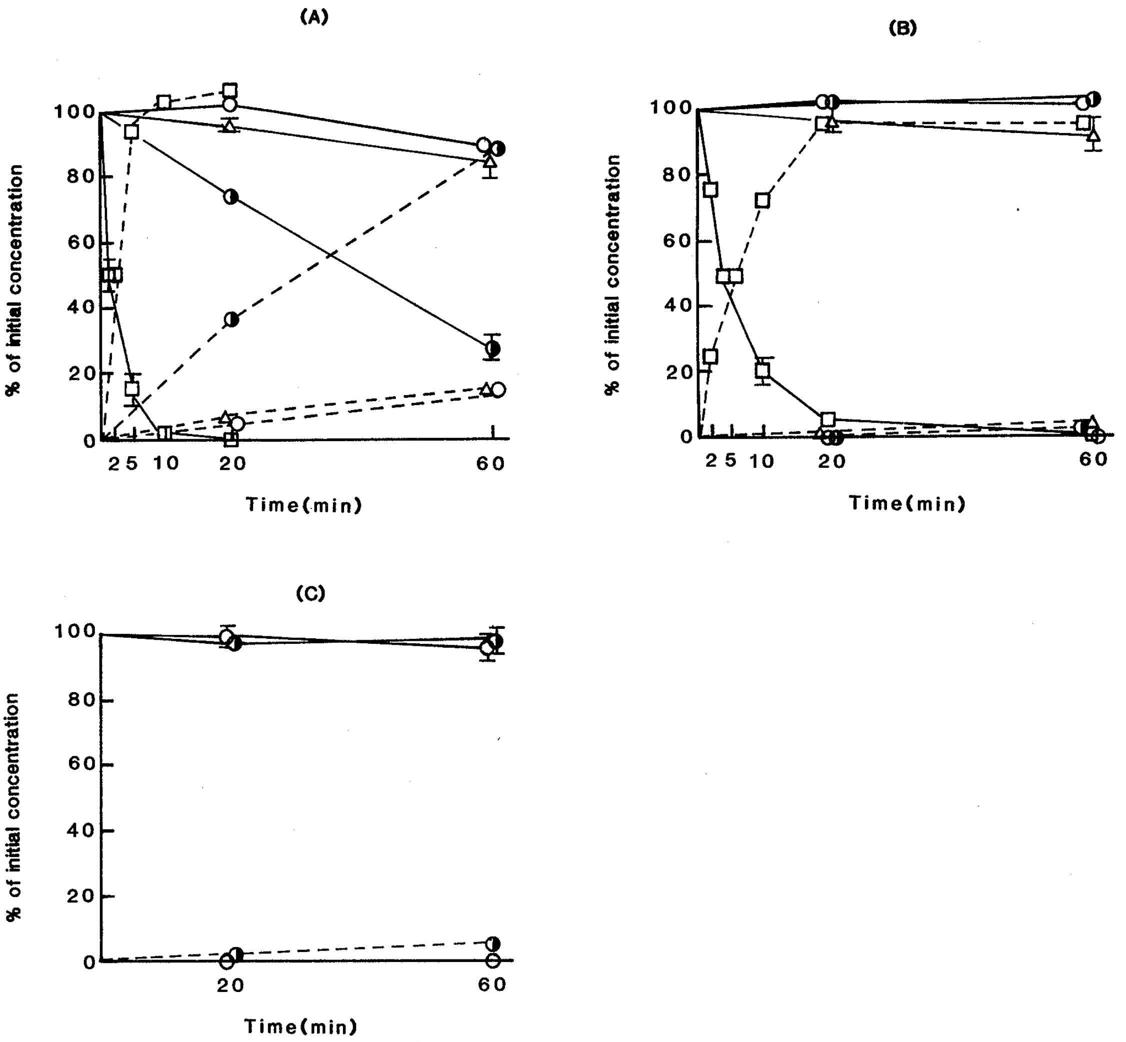

Fig. 5. The Decrease of Substrate (Solid Line) and the Increase of Product (Dashed Line) during Incubation with Skin (△), Plasma (○),[a] Plasma (◑)[b] and Liver (□) of Rat (Fig. A), Dog (Fig. B) and Human (Fig. C) *in Vitro*

Initial substrate concentration: 4 μm.
a) Crude enzyme extract 0.2 ml.
b) Crude enzyme extract 2.0 ml.

As shown in Fig. 3, most of the administered compound remained unchanged in the stratum corneum; the proportion of hydrocortisone butyrate, in which the propionyl group at the 21-position of hydrocortisone butyrate propionate had been hydrolyzed, was only 8.3%.

On the other hand, the rate of metabolism in the stratum corneum-removed skin was different from that in the stratum corneum. As shown in Fig. 4, significant quantities of hydrocortisone butyrate were found in the stratum corneum-removed skin, though unchanged hydrocortisone butyrate propionate was predominant.

As shown in Fig. 5, about 20% of hydrocortisone butyrate propionate was hydrolyzed in the rat after 60 min, while about 5% was hydrolyzed in the dog after 60 min. Species–specificity was recognized in plasma and liver.

Discussion

About 84% of hydrocortisone butyrate propionate remained unabsorbed on dog abdominal skin 5 h after administration, much larger than the amount, 56.4%, in rats.

Vickers[11] found that topically applied corticosteroid caused a vasoconstriction reaction for up to two weeks. While there was no vasoconstriction reaction either in the skin stripped by adhesive tape or in intact skin injected intradermally, they found that the stratum corneum is the main reservoir in the skin. Rougier *et al.*[12] reported that the stratum corneum plays an important role as a reservoir, because a linear relationship exists between the quantity of substance in this tissue and the total amount in the body and excreta.

Topical administration is thought to be safer than the systemic route with regard to systemic side effects, but in some cases, when used according to directions, corticosteroid can be absorbed percutaneously well enough to cause adrenocortical insufficiency.[13] Suzuki,[14] in his study of the distribution of corticosteroids and their side effects, found that the distribution is delocalized, without accumulation in a particular organ, and that the side effects are proportional to the drug potency. It should be noted that halogenated corticosteroids are poorly metabolized.

Recently, the mechanism of action of corticosteroids has been explained on the basis of an affinity to the steroid receptor followed by the synthesis of an anti-inflammatory protein.[15] Epsteine and Bonifas[16] and Khalid *et al.*[17] studied the receptor in human skin. It was believed that esterified steroid must be hydrolyzed to a free alcohol type for activity.[18,19] Muramatsu *et al.*[5] reported that hydrocortisone butyrate propionate increases the affinity of the corticosteroid receptor to hydrocortisone more than several fold. This suggests that the esterification of corticosteroid can affect not only the diffusion of corticosteroids in skin, but also the anti-inflammatory activity itself, through changes in the affinity of the corticosteroid receptor. To prevent systemic side effects, development of a corticosteroid which is metabolized rapidly after it acts strongly in the skin is desirable.[20]

It has become apparent that there are various kinds of enzymes in the skin; Hsia and Hao[21] found that hydrocortisone is metabolized to cortisone, Rawlins *et al.*[22] reported that the ester group of betamethasone 17-valerate is slightly hydrolyzed in human skin, Täuber and Toda[8] reported that the ester group of diflucortolone valerate at the 21-position is hydrolyzed by skin enzymes, and Herz-Hübner and Täuber[23] obtained a similar result with fluocortine valerate.

The authors studied the absorption, distribution, and metabolism of a diester derivative of hydrocortisone in rat skin, and found that most of the absorbed hydrocortisone butyrate propionate existing in the stratum corneum was the unchanged form.[7] This was also the case in the current study using dogs (Fig. 3). In the stratum corneum-removed skin, 80% of corticosteroid remained as metabolites in rats 5 h after the administration of hydrocortisone butyrate propionate, while about 50% was found in dogs (Fig. 4). Therefore, the esterase activity in this layer is stronger than in the stratum corneum, and rats have a stronger esterase activity than dogs.

As shown in Fig. 5, this species difference in esterase activity was observed not only in skin but also in plasma and liver, and the activity in the skin was nearly equal to that in plasma both for rats and dogs. This similaring is convenient from the standpoint of prevention of systemic side effects which are caused by esterified steroid after percutaneous absorption. Furthermore, it seems to be possible to estimate the rate of deesterification in human skin on the basis of the results found in plasma of the dog or human.

Täuber and Toda[8] examined the metabolism of diflucortolone valerate in the skin and reported that rats and guinea pigs have a stronger activity than humans. The weak metabolic activity found in the dog is therefore useful in order to estimate the pharmacokinetics and the effects of esterified corticosteroid administered percutaneously.

Acknowledgment The authors are particularly indebted to the late Dr. M. Washitake (Research Center, Taisho Pharmaceutical Co., Ltd.) for his useful suggestions, and they also wish to thank Mr. T. Yamahira for carrying out some of the determinations.

References

1) A. W. McKenzie and R. M. Atkinson, *Arch. Dermatol.*, **89**, 741 (1964).
2) T. Kobayashi, *Nishinippon Hifuka*, **36**, 360 (1974).
3) A. Kukita and S. Harada, *Nishinippon Hifuka*, **44**, 657 (1982).
4) A. Pannatier, P. Jenner, B. Testa and J. C. Etter, *Drug Metab. Rev.*, **8**, 319 (1978).
5) M. Muramatsu, M. Tanaka, S. Otomo, H. Aihara and K. Kuriyama, *Jpn. J. Pharmacol.*, **37**, 143 (1985).
6) T. Misaki, M. Yamada, R. Hirai, M. Komori, M. Washitake, Y. Ozawa, I. Koyama and H. Yoshizawa, *Yakuzaigaku*, **42**, 92 (1982).
7) M. Washitake, Y. Ozawa, I. Koyama, T. Misaki, M. Yamada, R. Hirai and H. Yoshizawa, *Yakuzaigaku*, **42**, 84 (1982).
8) U. Täuber and T. Toda, *Arzneim.-Forsch.*, **26**, 1484 (1976).
9) K. Soda, M. Mitsukuchi, J. Nakagami, Y. Tachi, J. Sawada, S. Otomo and M. Ohzeki, *Yakugaku Zasshi*, **102**, 365 (1982).
10) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
11) C. F. H. Vickers, *Arch. Dermatol.*, **88**, 72 (1963).
12) A. Rougier, D. Dupuis, C. Lotte, R. Roguet and H. Schaefer, *J. Invest. Dermatol.*, **81**, 275 (1983).
13) S. Shimao, *Nippon Hifuka Gakkai Zasshi*, **89**, 61 (1979).
14) M. Suzuki, *Nippon Hifuka Gakkai Zasshi*, **94**, 831 (1984).
15) S. Tsurufuji, K. Sugio and F. Takenaka, *Nature* (London), **280**, 408 (1979).
16) E. H. Epsteine and J. M. Bonifas, *J. Invest. Dermatol.*, **78**, 144 (1982).
17) B. A. K. Khalid, S. Gyorki, G. L. Warne and J. W. Funder, *Clin. Endocrinol.*, **18**, 407 (1983).
18) J. Tamm and K. Vogt, *Acta Endocrinol.*, **35**, 75 (1960).
19) E. J. Collins, J. Aschenbrenner and M. Nakahama, *Steroids*, **20**, 543 (1972).
20) H. J. Lee and M. R. I. Soliman, *Science*, **215**, 989 (1982).
21) S. L. Hsia and Y. Hao, *Steroids*, **10**, 489 (1967).
22) M. D. Rawlins, V. Skaw and S. Shuster, *Brit. J. Pharmacol.*, **66**, 441 p (1979).
23) U. Herz-Hübner and U. Täuber, *Arzneim-Forsch.*, **27**, 2226 (1977).