[Chem. Pharm. Bull. 20(3)589—592(1972)]

UDC 547.92.03.04 : 615.32.011.4.011.5

Determination of Dexamethasone and Related Compounds with Isoniazid Dihydrochloride and Its Application to Pharmaceutical Analysis

Tsunetoshi Kaito, Koji Kasuya and Miyako Yokota

Research Laboratory, Taisho Pharmaceutical Co., Ltd.[1]

(Received June 24, 1971)

A number of spectrophotometric methods based on the reaction of Δ^4-3-keto-, $\Delta^{1,4}$-3-keto- and 17, 21-dihydroxy-20-keto-steroids have been applied for the determination of dexamethasone by the use of various reagents such as *p*-aminodimethylaniline hydrochloride stannous chloride,[2] 4-aminoantipyrine hydrochloride,[3] 2,6-di-*tert*-butyl-*p*-cresol,[4] isoniazid,[5] diethyl oxalate,[6] tetrazolium,[7] sodium borohydride[8] and phenylhydrazine hydrochloride.[9] The isoniazid (INH) method for Δ^4-3-ketosteroids reported by Umberger[5] has been suitable for the analysis of hydrocortisone and methyltestosterone in pharmaceutical preparations. However, in the case of a steroid having $\Delta^{1,4}$-3-keto group, the reaction was very slow and fairly different from that of Δ^4-3-ketosteroid. The yellow color due to formation of hydrazone from INH and Δ^4-3-ketosteroid is developed smoothly only when the reagent is used with hydrochloric acid of constant molar ratio of two to one against INH. In this respect, for $\Delta^{1,4}$-3-ketosteroid more severe control of the reaction condition seems to be needed. The coloration was easily influenced by the comsumption of hydrochloric acid with basic impurities in sample. Consequently, the reproducible results were not often obtained. In the present paper, isoniazid dihydrochloride (INH.2HCl) instead of INH as a reagent is used. The INH.2HCl, in comparison with INH, appeared to be useful for the analysis of $\Delta^{1,4}$-3-ketosteroids in pharmaceutical mixtures. In addition, a procedure for separation of dexamethasone and related hydrocortisone using elution column chromatography on silicic acid was also established.

Experimental

Materials——(a) INH. 2HCl was prepared from INH by the usual method using dry HCl gas. (b) 0.2% (w/v) INH. 2HCl solution: To a solution of INH. 2HCl (200 mg) in MeOH (90 ml) was added concentrated HCl (0.075 ml) and the solution was adjusted to 100 ml with MeOH. (c) Silicic acid: Mallinckrodts's analytical reagent (100 mesh). (d) All materials were of reagent grade.

General Separation of Dexamethasone——A Pharmaceutical sample containing 500—2500 μg of dexamethasone was transferred into a 50 ml of centrifuge tube and extracted with three 20 ml portions of petroleum ether and the extracts were discarded. The residue was reextracted with four 20 ml portions of MeOH and the MeOH extracts were combined and evaporated in order to eliminate H_2O contained in them. The

1) Location: *3-34-1, Takada, Toshima-ku, Tokyo.*
2) R. Hüttenrauch, *Z. Physiol. Chem.*, **326**, 166 (1961).
3) E.P. Schulz, M.A. Diaz, G. Lopez, L.M. Guerrero, H. Barrera, A.L. Pereda and A. Aguillera, *Anal. Chem.*, **36**, 1624 (1964).
4) *a*) E.P. Schulz and J.D. Neuss, *Anal. Chem.*, **29**, 1662 (1957); *b*) S. Ansari and R.A. Khan, *J. Pharm. Pharmacol.*, **12**, 122 (1960); *c*) E.P. Schulz, M.A. Diaz and L.M. Guerrero, *J. Pharm. Sci.*, **53**, 1119 (1964).
5) E.P. Umberger, *Anal. Chem.*, **27**, 768 (1955).
6) S. Görög, *Anal. Chem.*, **42**, 560 (1970).
7) *a*) W.J. Mader and R.R. Buck, *Anal. Chem.*, **24**, 666 (1952); *b*) F.M. Kunze and J.S. Davis, *J. Pharm. Sci.*, **53**, 1170, 1259 (1964).
8) *a*) S. Görög, *Steroids*, **11**, 93 (1968); *b*) S. Görög, *J. Pharm. Sci.*, **57**, 1737 (1968).
9) *a*) R.H. Silber and C.C. Porter, *J. Biol. Chem.*, **185**, 201 (1950); *b*) M.L. Lewbart and V.R. Mattox, *Anal. Chem.*, **33**, 559 (1961).

residue was dissolved in MeOH and the solution was diluted to contain 10—30 μg/ml of dexamethasone with MeOH for analysis.

Chromatographic Separation of Dexamethasone——A mixture of silicic acid (20 g) and H_2O (4 ml) was added to $CHCl_3$ (30 ml) and prepared slurry and the srurry was transferred into a column (20×400 mm). A pharmaceutical sample containing 250—2000 μg of dexamethasone was mixed with twice amount of silicic acid and, with water of the same proportion to silicic acid as used in the column preparation. The mixture was carefully added to the top of the silicic acid column and was then chromatographed by using $CHCl_3$ as the first eluent at a flow rate of 0.5—1.0 ml/min. After 150 ml of $CHCl_3$ was passed through the column, the eluting solvent was changed to 3% (v/v) EtOH in $CHCl_3$.[10] First 20 ml of the eluates was discarded and next 100—120 ml of the eluates was collected and evaporated. The residue was dissolved in MeOH and the solution was diluted to contain 10—30 μg/ml of dexamethasone with MeOH for analysis.

Analytical Procedure——To 5 ml of the test solution containing 10–30 μg of $\Delta^{1,4}$-3-ketosteroid in MeOH was added 5 ml of 0.2% (w/v) INH. 2HCl solution and the reaction mixture was allowed to stand for 2 hr at room temperature or heated in a water bath for 40 min at 40°. After cooling, the absorbance of the solution was measured against the reagent blank at 407 mμ.

Result and Discussion

Comparisons of the relative intensities of dexamethasone obtained by using the INH and the INH.2HCl at room temperature are shown in Fig. 1. When INH and hydrochloric acid were used with the ratio of about one mole of INH to two mole of concentrated hydrochloric acid, that is, a 0.05 (w/v) to 0.0625 (v/v), the sensitivity reached to a maximum. On the contrary, the use of the INH.2HCl with or without addition of hydrochloric acid gave fairly stable intensity and the color development was not greatly influenced by changes of the molar ratio. It was necessary to obtain maximum intensity to use the INH.2HCl solution of 0.1% (w/v) or more which contains hydrochloric acid. For the standard procedure, 0.2% (w/v) INH·2HCl was employed. The concentration of concentrated hydrochloric acid in the reagent was varied between 0.025 and 0.35% (v/v), other analytical conditions remaining unchanged, at room temperature (Fig. 2). The optimum absorbance of dexamethasone was observed by the use of 0.06—0.08% (v/v) concentrated hydrochloric acid. Therefore, 0.075% (v/v) concentrated hydrochloric acid was chosen. The effect of the reaction time and temperature on the color intensity are shown in Fig. 3. The time required for the optimum color development was 2 hr at room temperature and 40 min at 40°. No significant difference was observed in the absorbance of solution between these conditions in the coloration.

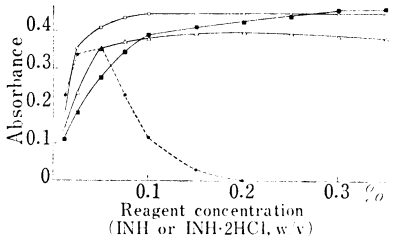

Fig. 1. Effect of INH or INH. 2HCl Concentration on Color Development

---●---: INH in MeOH which contains 0.0625% (v/v) concentrated HCl
—△—: molar ratio of HCl to INH is kept 2:1 in MeOH
—○—: INH. 2HCl in MeOH which contains 0.075% (v/v) concentrated HCl
—■—: INH. 2HCl in MeOH
final concentration of dexamethansone 10 μg/ml

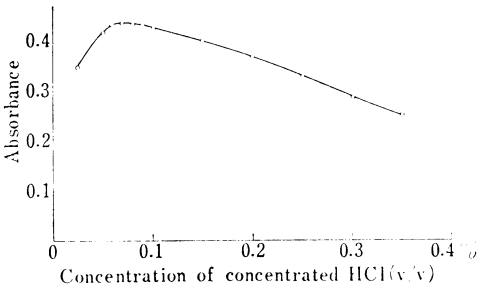

Fig. 2. Effect of HCl Concentration in 0.2%(w/v) INH. 2HCl on Color Development
final concentration of dexamethasone 10 μg/ml

10) EtOH is usually contained in commercial $CHCl_3$ as a stabilizer (about 1%) and then practical amount of EtOH in $CHCl_3$ is a little greater.

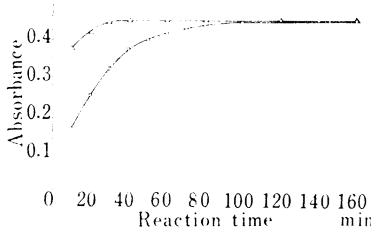

Fig. 3. Effect of Reaction Time and Temperature on Color Development
—○—: room temperature
—△—: 40°
final concentration of dexamethasone 10 μg/ml

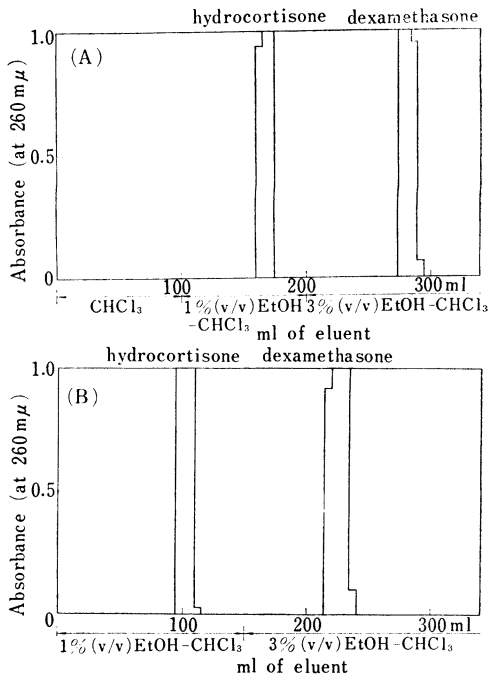

Fig. 4. Typical Separation of Hydrocortisone and Dexamethasone on Silicic Acid Column
silicic acid (20 g) containing H_2O (4 ml)
hydrocortisone: 2 mg; dexamethasone: 2 mg

In connection with the problem of the pharmaceutical analysis, a separation of steroid from some components was sometimes required prior to assay. Bracey and his coworkers[11] reported column chromarography using celite for the determination of hydrocortisone and related dexamethasone. This method of separation was not satisfactorily applied for the determination of dexamethasone which is usually solubilized in a micelle. There was a loss of about 15% through adsorption on the column. In the procedure herein described, a chromatographic separation of dexamethasone was achieved by use of silicic acid with water as adsorbent. The amount of water adsorbed on silicic acid was kept at 20% with respect to silicic acid from the preliminary works. This proportion appeared to be suitable for the separation of dexamethasone from ingredients. As snown in Fig. 4, dexamethasone can be easily separated from hydrocortisone by EtOH and $CHCl_3$ as eluents, whereas both steroids can not be readily eluted with $CHCl_3$ alone under these conditions. The separation of hydrocortisone from pharmaceutical mixtures was also efficiently

Table 1. Spectral Data of Some Ketosteroids after Treatment with INH. 2HCl Reagent

Compounds	λmax mμ	ε_{max} a)
$\Delta^{1,4}$-3-ketosteroid		
Dexamethasone	407	17300
Predonisolone	407	16300
Androsta-1,4,-dien-3,17-dione	407	14900
Androsta-1,4-dien-3,11,17-trione	407	15700
17-Hydroxy-androsta-1,4-dien-3-one acetate	407	16200
17-Dihydroxy-pregna-1,4-dien-3,11, 20-trione	407	17400
Δ^{4}-3-ketosteroid		
Hydrocortisone	380	11900
Methyltestosterone	380	12400

a) apparent molar absorption coefficient

11) A. Bracey, L. Garrett and P.J. Weiss, *J. Pharm. Sci.*, **55**, 1113 (1966).

achieved by these methods. The optimum eluting solvent was 1% (v/v) EtOH in $CHCl_3$ for hydrocortisone and 3% (v/v) EtOH in $CHCl_3$ for dexamethasone.

Table I summarizes the apparent molar absorption coefficients and the absorption maxima in some Δ^4- and $\Delta^{1,4}$-3-ketosteroids. Concerning $\Delta^{1,4}$-3-ketosteroids, the absorbancy obtained with INH.2HCl reagent was larger than that with INH reagent. The results of the analyses of dexamethasone and hydrocortisone in some formulations are shown in Table II. The results are quite satisfactory. The combination of the column chromatography-colorimetry procedures could be generally applicable in various types of pharmaceutical preparations.

TABLE II. Results of Ketosteroids in Some Formulations determined by the Present Method

Steroids (formulation)	Nominal (%)	Found[a] (%)	Standard deviation (%)
Dexamethasone (ointment)	0.05	0.051	±1.2
Dexamethasone (cream)[b]	0.025	0.0252	±0.9
Dexamethasone (lotion)	0.05	0.052	±1.1
Hydrocortisone (suppository)[b]	0.05	0.050	±1.0

a) mean value of five determinations
b) Separation of steroid was carried out by using the column chromato graphy. Hydrocortisone was treated with the method indicated in Fig. 4 (A).

Acknowledgement The authros express their sincere gratitude to Prof. A. Tsuji, University of Showa, for his valuable discussions and advices in this work and indebted to S. Uehara, Vice President of this company, for his permission to publish this paper. Their thanks are also due to Dr. S. Ikawa, director of scientific relations department and to Dr. I. Tanaka, director of research laboratory, of this company, for their continuous encouragement.

[Chem. Pharm. Bull. 20(3) 592—596 (1972)]

UDC 547.551.42.04 : 547.398.1.057

Benzodiazepines. VI.[1] A Rearrangement of 2-Aminoacetanilides to Anilinoacetamides

KIKUO ISHIZUMI, SHIGEHO INABA and HISAO YAMAMOTO

Pharmaceuticals Division, Sumitomo Chemical Co., Ltd.[2]

(Received June 26, 1971)

In addition to the expected 7-nitrobenzodiazepin-2-one (**2**), we have isolated a small amount of 2-(2-benzoyl-4-nitroanilino)-N-methylacetanilide (**5**) as a by-product from the chromic acid oxidation of the 2-aminomethyl-5-nitroindole (**1**).[1] It has been postulated that the conversion of **1** to **5** involves, initially oxidative opening of **1** to the intermediate **3**, which then rapidly rearranges to **5**. Rearrangement is thought to proceed through the cyclic transition state (**4**) by intramolecular nucleophilic attack of the amino group on the

1) Part V: S. Inaba, K. Ishizumi, K. Mori and H. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 722 (1971).
2) Location: *2-1, Takatsukasa-4-chome, Takarazuka-shi, Hyogo.*