proteins. These results show that entrapment of drug could resemble a drug-protein interaction at a sufficiently low ratio of K_1 to K_2. This results from the decrease in drug available for diffusion from V_2 to the external sink. At low values of the K_1 to K_2 ratio, as drug is removed from V_2, it is not immediately replaced by drug from within the erythrocytes; the end result is the same as if the drug had actually been bound by the erythrocyte.

Dynamic dialysis may be a useful method for the study of drug entrapment since equilibrium methods cannot be used to determine transport rate constants of drug out of erythrocytes. However, it is important not to confuse drug entrapment by erythrocytes with true drug–erythrocyte complexation.

REFERENCES

(1) M. C. Meyer and D. E. Guttman, *J. Pharm. Sci.*, **57**, 1627 (1968).

(2) C. A. Cruze and M. C. Meyer, *ibid.*, **65**, 33 (1976).

(3) L. S. Schanker, P. A. Nafpliotis, and J. M. Johnson, *J. Pharmacol. Exp. Ther.*, **133**, 325 (1961).

(4) J. N. McArthur, P. D. Dawkins, and M. J. H. Smith, *J. Pharm. Pharmacol.*, **23**, 32 (1971).

(5) D. Lester, G. Lolli, and L. A. Greenberg, *J. Pharmacol. Exp. Ther.*, **87**, 329 (1946).

(6) P. K. Smith, H. L. Gleason, C. G. Stoll, and S. Ogorzalek, *ibid.*, **87**, 237 (1946).

(7) M. H. Bickel, *J. Pharm. Pharmacol.*, **27**, 733 (1975).

(8) R. C. Baselt and C. B. Stewart, *Res. Commun. Chem. Pathol. Pharmacol.*, **15**, 351 (1976).

(9) S. P. Colowick and F. W. Womack, *J. Biol. Chem.*, **244**, 774 (1969).

(10) H. Sund, in "New Techniques in Amino Acid, Peptide, and Protein Analysis," A. Niederwieser and G. Pataki, Eds., Ann Arbor Science Publishers, Ann Arbor, Mich., 1971, pp. 405–411.

(11) J. G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," Drug Intelligence, Hamilton, Ill., 1971, p. 268.

(12) M. C. Meyer and D. E. Guttman, *J. Pharm. Sci.*, **57**, 895 (1968).

(13) D. L. Parsons and J. J. Vallner, *ibid.*, **67**, 1344 (1978).

ACKNOWLEDGMENTS

Presented at the APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1978.

D. L. Parsons thanks the American Foundation for Pharmaceutical Education for support. The authors express sincere thanks to Ms. J. Cole for her help in manuscript preparation.

High-Performance Liquid Chromatographic Separation and Identification of Epimeric 17-Ketone Impurities in Commercial Sample of Dexamethasone Sodium Phosphate

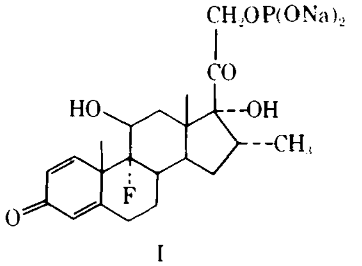
ERIC C. JUENGE ˣ **and JAMES F. BROWER**

Received July 12, 1978, from the *National Center for Drug Analysis, Food and Drug Administration, St. Louis, MO 63101.* Accepted for publication October 3, 1978.

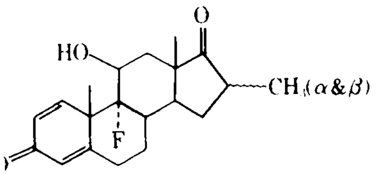
Abstract ☐ A commercial sample of dexamethasone sodium phosphate solution for injection was found to contain 56% of the label concentration and to be extensively contaminated (~50%) with a white insoluble solid, which was identified as a mixture of the 16α- and 16β-methyl epimers of 9-fluoro-11β-hydroxy-16-methylandrosta-1,4-diene-3,17-dione. High-performance liquid chromatography (HPLC) was used to separate, identify, and quantitate these epimers and to determine their presence in commercial samples. One epimer was identified by HPLC comparison with a synthesized specimen of 9-fluoro-11β-hydroxy-16α-methylandrosta-1,4-diene-3,17-dione. The second peak was identified as the 16β-epimer by epimerization of the synthesized α-component with alkali to obtain a product whose chromatogram matched that of the impurity. These conclusions are supported by data obtained by IR and UV spectrophotometry, TLC, and the blue tetrazolium test.

Keyphrases ☐ Dexamethasone sodium phosphate—analysis, high-performance liquid chromatography, identification of impurities in a commercial sample, epimerization ☐ High-performance liquid chromatography—analysis of dexamethasone sodium phosphate and impurities in a commercial sample ☐ Epimerization—dexamethasone sodium phosphate in a commercial solution

Drug samples contain various impurities: drug intermediates, by-products, and degradation products introduced during manufacture, packaging, or storage, as well as products of the interaction of such substances with excipients in the sample or with the drug itself. These impurities constitute physiological and mechanical hazards. For example, during degradation, the structure of the compound may retain active or modified active centers that, through structural relationship to the intact drug, may bestow undesirable physiological activity or toxic properties on the decomposition product. An example of a physical or mechanical hazard is the presence or formation of insoluble substances during intravenous injection.



I



II

0022-3549/79/0500-0551$01.00/0

A sample of dexamethasone sodium phosphate [9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-(dihydrogen phosphate) disodium] (I) solution for injection was found to contain a suspended white solid. After examination by high-performance liquid chromatography (HPLC), TLC, UV and IR spectrophotometry, thermal microscopy, and reaction with blue tetrazolium, the solid was identified as a mixture of the 16α- and 16β-methyl epimers of 9-fluoro-11β-hydroxy-16-methylandrosta-1,4-diene-3,17-dione (II).

EXPERIMENTAL

Materials—Dexamethasone and dexamethasone sodium phosphate NF reference standards were used. Reagent grade chloroform, methylene chloride, methanol, acetone, hexane, squalane, sodium bicarbonate, sodium sulfite, tetrabutylammonium hydroxide, anhydrous magnesium sulfate, acetic acid, and concentrated hydrochloric acid were used as received.

Dexamethasone acetate[1] was checked by melting point and comparison of its IR spectrum with a published spectrum (1). Sodium bismuthate analytical reagent[2] (assay for $NaBiO_3$, 80.0–85.0%) was used as obtained. A reagent grade solution of sodium hydroxide[3] (50% by weight) was used for the epimerization work.

Instruments—A grating IR spectrophotometer[4] (mineral oil mulls on sodium chloride plates), a UV/near-IR spectrophotometer[5] (UV spectra obtained from solutions in methanol), and a high-performance liquid chromatograph[6] were used. All melting points were taken by thermal microscopy with a microscope[7] and a Kofler melting-point stage.

HPLC—Instrument parameters included a microparticulate, reversed-phase column[8], 3.9 mm × 30 cm; a 50% methanol–water mobile phase; and a flow rate of 2.0 ml/min. Retention times, in terms of the distance from injection to peak appearance on the chart paper in concomitant runs, were converted to capacity factors (2).

Sublimation—Sublimation was effected by placing crude material in a 0.5-ml sublimation beaker, covered with a watch glass, directly on a hot stage maintained within 230–240°.

TLC—A limited study was made with TLC strips or plates, 6–7 cm long, by upward development in an equilibrated chamber. Chromatographic sheets[9], high-performance plates[10], and reversed-phase plates[10] were used. All thin-layer materials contained a fluorescent indicator. Spots were visually located by placing the plates or strips under shortwave UV light.

Preparation of 9-Fluoro-11β-hydroxy-16α-methylandrosta-1,4-diene-3,17-dione (II*a*)—Following the procedure of Rausser and Oliveto (3), 240 mg of dexamethasone was oxidized by sodium bismuthate; 140 mg of product should have been obtained (3). However, yields of only 22–30 mg were consistently obtained. The reduced yield was attributed to the insolubility of the product in the highly dilute acetic acid solution and could be improved by modifications described under *Results and Discussion*.

The synthetic 17-ketone (II*a*) was dissolved in methanol and examined by HPLC. A capacity factor (2) of 8.01 was observed, and the areas of extraneous peaks in the chromatogram were estimated as being less than 1% of the area of the major component.

Epimerization of II*a* to 16-Methyl Epimeric Mixture (II) Favoring β-Isomer—To 15 mg of the 17-ketone (II*a*) was added 1 ml of anhydrous methanol and 0.5 ml of 50% aqueous sodium hydroxide, resulting in a pale-orange solution. The mixture was stirred at room temperature for 10 min, followed by addition of 5 ml of distilled water, which caused precipitation of a white solid. The solid (10 mg) was collected, washed three times with distilled water, and allowed to dry overnight.

The solid was subjected to thermal microscopy (2°/min), which gave sintering at 136°, initial needle formation at 222° with the entire mass laced with needles at 239°, and melting at 250–254° to an orange liquid. The material was dissolved in methanol and examined by HPLC. Capacity factors (2) of 7.96 and 8.97, which correspond to a relative retention α (2) of 1.13, were observed. The areas of extraneous peaks in the chromatogram were estimated as being less than 1% of those of the major components.

Description and Preparation of Sample—The vials contained a white precipitate in a pale-yellow solution of pH 7.2–7.3. The contents of each drug vial, 5 ml, labeled to indicate 4 mg of dexamethasone phosphate (equivalent to 4.4 mg of dexamethasone sodium phosphate)/ml, were extracted three times with a total of 20 ml of chloroform. The separated chloroform layers were combined and then washed with 10 ml of deionized water containing 1 drop of 50% NaOH, followed by a wash with 15 ml of deionized water. The chloroform was removed by evaporation under a nitrogen flush while the mixture was warmed gently on a steam bath.

The residue was dissolved in methanol for UV examination (λ_{max} 240 nm) and HPLC study (capacity factors 7.93 and 8.92, relative retention 1.12). If treatment with the dilute alkali wash was omitted, the same HPLC peaks with the same relative peak heights were obtained, but paraben (preservative) peaks also appeared. The paraben-free residue was dried over phosphorus pentoxide overnight, leaving a crude white solid, mp 249–252° dec. Observed with a melting-point stage (1°/min), rapid sublimation began at 223° and colorless needles developed from the residual solid; as these needles melted, the mass laced with needles became an orange melt. Some sintering with formation of orange spots occurred during melting.

The residue isolated from the sample was soluble in chloroform, methanol, and acetone and was insoluble in ether, hexane, water, and dilute aqueous sodium hydroxide. It was recrystallized from acetone–hexane by dissolving the material in acetone and adding hexane in small portions while boiling until turbidity developed. On cooling in an ice bath, crystals separated immediately and were collected and washed with cold hexane–acetone (3:1) to give a white solid, mp 246–250°.

A solution of the crude impurity in methanol did not reduce blue tetrazolium[11]. Therefore, the impurity contained neither free dexamethasone nor other steroidal α-ketols.

UV spectra of all materials were examined just before analysis by HPLC to assure that a maximum absorbance appeared at 240 nm. This precaution was necessary because these materials decomposed under certain conditions; a methanolic solution of the drug impurity exhibited maximum absorbance at 253 nm after storage in a clear glass container for about 1 month.

RESULTS AND DISCUSSION

The synthesized α-epimer (II*a*), the synthesized epimeric mixture (II), and the impurity from the sample gave spectra that, in the IR carbonyl region (1600–1800 cm^{-1}) and UV region (λ_{max} 240 nm), were identical. The IR spectrum of II closely matched that of the impurity; both spectra showed peaks at 1744, 1770, 1630, 1615, 1238, 1010, and 920 cm^{-1}. The IR spectrum of the α-epimer closely matched the major peaks reported for that compound by Gut (4).

Two modifications were devised to recover additional adsorbed product (α-epimer) from the spent sodium bismuthate oxidant. First, in a method similar to one used by Gut (4), the solids collected by filtration were treated with aqueous sodium sulfite and concentrated hydrochloric acid until they dissolved, and the chloroform extraction procedure was repeated on the solution. This procedure yielded an additional 160–175 mg of product. Alternatively, it was found that the solids could be dried in air and then extracted with acetone in a soxhlet apparatus. After the extract was dried with anhydrous magnesium sulfate and the acetone was removed on a steam bath, this modification yielded an additional 170–190 mg of product. The latter procedure is preferred, because it avoids any possibility of halogenation.

The batches of II*a* obtained by these procedures were examined by thermal microscopy (1°/min) and gave a melting point of 244–247° [lit. (4) mp 245–249°]; practically all material passed quickly into the melt; a few crystals lingered to the higher temperature range, suggesting the occurrence of thermal epimerization. Hardly any sublimation was observed.

The 17-keto impurity was determined quantitatively by extracting a new vial of the sample injection with chloroform, evaporating the chloroform, dissolving the residue in methanol, and injecting the methanolic

---

[1] Schering Corp., Kenilworth, NJ 07033.

[2] Mallinckrodt Chemical Works, St. Louis, MO 63160.

[3] Taylor Chemical Co., St. Louis, MO 63144.

[4] Model 337, Perkin-Elmer Corp., Norwalk, CT 06856.

[5] Model ACTA MIV, Beckman Instruments, Fullerton, CA 92634.

[6] Model 3500B, Spectra-Physics, Santa Clara, CA 95051.

[7] Carl Zeiss, New York, NY 10018.

[8] μBondapak C_{18}, Waters Associates, Milford, MA 01757.

[9] Chromagram, silica gel 6060 and alumina 6063, Eastman Organic Chemicals Division, Eastman Kodak Co., Rochester, NY 14650.

[10] QHPF and RPF Nano/gram, Quantum Industries, Fairfield, NJ 07006.

[11] Sigma Chemical Co., St. Louis, MO 63178.

Table I—TLC of Drug Impurity, 16α-Methyl-17-ketone (IIa), Epimeric Product from 16α-Methyl-17-ketone, and Dexamethasone Acetate

Adsorbent	Solvent System[a]	R_f[b]
Silica gel	Methanol–water	0.80
	Butyl acetate	0.62
Alumina	Methanol–water	0.81
Alumina with 2% squalane[c]	Methanol–water	0.62
Reversed phase	Methanol–water	0.00
	Butyl acetate	0.92
	Acetonitrile–water	0.45
High-performance silica gel	Methanol–water	0.80

[a] Mixed solvents were used in 1:1 proportions. [b] Single values given because all compounds gave identical R_f values in each system. [c] Each plate was dipped twice in 2% squalane in *n*-pentane just before use.

solution into the chromatograph. The reference standard used was a portion of the dried residue previously isolated from a chloroform extract of three other vials and examined by IR and TLC. The amount of 17-keto impurity found was 1.4 mg/ml (corresponding to 2.2 mg of dexamethasone sodium phosphate/ml) and was calculated by use of the larger of the two peaks in the epimeric mixture. Analysis for dexamethasone sodium phosphate by the USP XIX procedure (5) gave 2.45 mg of dexamethasone sodium phosphate/ml, 56% of the stated value (4.4 mg/ml). The amount of impurity thus calculated accounts for the shortage and suggests oxidation of a dexamethasone derivative as the source of the impurity.

Primary TLC screening consistently suggested that the white, water-insoluble precipitate in the dexamethasone sodium phosphate sample was comprised of a single component and that it was dexamethasone acetate. This latter compound generated R_f values identical with those of the impurity from the sample in various systems (Table I). In most cases, aqueous methanol was used as the developing solvent since this solvent was used in HPLC. Two adsorbents, reversed-phase precoated plates[10] and squalane on alumina, were used to approximate the reversed-phase conditions provided by the high-resolution HPLC column[8]. In reference to thermal microscopy of dexamethasone acetate, Ref. 6 notes that rods develop from the residual crystals during the melting-point determination; this phenomenon also was observed in this laboratory when the melting point of the impurity was determined with a melting-point stage.

Despite these data, the IR spectrum of dexamethasone acetate was distinctly different from that of the impurity. Dexamethasone acetate displayed two sharp carbonyl peaks at 1730 and 1750 cm^{-1}, whereas the impurity showed only the peak at 1744 cm^{-1}. In addition, when a mixture of dexamethasone acetate and the impurity was examined, a depressed melting point was observed. HPLC on the reversed-phase column demonstrated that the impurity consisted of two components, which eluted as a single peak when 40% acetonitrile was used as the mobile phase but separated into two peaks when 50% methanol was employed. This result confirmed that the impurity was not dexamethasone acetate, which had a much larger capacity factor (13.80).

An attempt was made to separate the epimers by sublimation. Long, fine, single needles grew from the material up toward the watch glass. These long needles were easily separated from the remaining material and melted at 257–259°; no signs of sintering (orange coloration) before melting were noted. A smaller amount of material, including some fine short needles, mp 237–257°, collected on the watch glass.

TLC of the crude, recrystallized, and sublimed (mp 257–259°) materials on silica gel gave spots with the same R_f when the chromatogram was developed with methanol-water (1:1). HPLC demonstrated that the sublimate was still a mixture, now containing a majority of the epimer that eluted last. Recrystallization gave a product that contained approximately equal amounts of the epimers.

The integrity of the steroidal A ring in the impurity was indicated by absorption in the UV region. Both Δ^4- and $\Delta^{1,4}$-3-ketone moieties in the A ring show absorption at 240 nm (7). The observed shift of the UV wavelength of maximum absorbance in aged methanolic solutions of the impurity, stored in closed, clear glass containers, probably resulted from a photochemical transformation at the A ring of the steroid. Prednisolone acetate, for example, can undergo such photocatalyzed transformations at the $\Delta^{1,4}$-3-keto group, leading to other compounds that absorb in the UV region (8). The IR spectrum of the impurity was also similar to that obtained for steroids with A rings that contain an α,β-unsaturated carbonyl function and showed absence of bands characteristic of the -CH_2OPO_2O- group found in dexamethasone phosphate or its sodium salt.

The negative blue tetrazolium reaction for steroidal α-ketols suggested that cleavage had occurred at the C_{17} position. Cleavage may result from oxidation at the C_{17} side chain, and the possible products from such a reaction—aldehydes, acids (such as etianic acids), and the 17-ketone—were discussed by Cohen in his review on dexamethasone (7). Of these candidates, the IR examination appeared to eliminate aldehydes. Furthermore, it seemed likely that the acidic compounds could be eliminated from consideration, since they would be expected to elute rapidly from the lipophilic HPLC column or to be removed in the sample preparation steps when the chloroform extracts were washed with dilute aqueous alkali. Thus, the 17-ketone was the most likely candidate.

To confirm this deduction, an independent synthesis of the 17-ketone analog of dexamethasone by the method of Rausser and Oliveto (3) was undertaken. Because only minute quantities of impurity were available, it was decided to use direct syntheses of the 16α- and 16β-methyl components of the impurity as reference standards instead of fractional crystallization, sublimation, or isolation and identification by HPLC; since the compounds were not completely resolved, the latter technique would have required careful timing during the collection of the eluted fractions.

HPLC (Fig. 1, curve 2) of the synthesized 17-ketone (II*a*) gave a single peak and showed a retention time identical with that of the first peak (Fig. 1, curve 1) in the chromatogram of the impurity. This match established the first, larger peak in the chromatogram of the impurity as the one representing the α-epimer of II, since there is no reason to expect inversion or even racemization at the C_{16} position of dexamethasone after oxidation with sodium bismuthate.

An epimerization of the 16α-methyl-17-ketone (II*a*) was devised to generate the epimeric 16-methyl mixture of the 17-ketone (II), and these epimers showed retention times in HPLC (Fig. 1, curve 3) that were identical with those of the two components in the impurity of the drug sample. The IR spectrum of the impurity closely matched that of the epimeric mixture of the 17-ketone obtained by alkali treatment of the 16α-methyl optical isomer. TLC consistently gave spots with the same R_f values (Table I) for the synthesized 16α-methyl isomer of the 17-ketone (II*a*), the epimerized ketone (II), and the impurity, and the epimers did not separate with the chromatographic systems used.

The synthesized α-epimer showed less tendency to sublime in the mass during melting-point determination than did the impurity containing the β-epimer. The sublimed long needles growing from the impurity were higher melting and were shown by HPLC to be predominantly the β-epimer. Some sublimate, which collected on the watch glass cover of the sublimation apparatus, melted at a lower temperature but showed no change in the ratio of the HPLC peaks when compared to the original impurity and may have suffered slight decomposition during sublimation. The long needles appeared to collect at a higher temperature near the

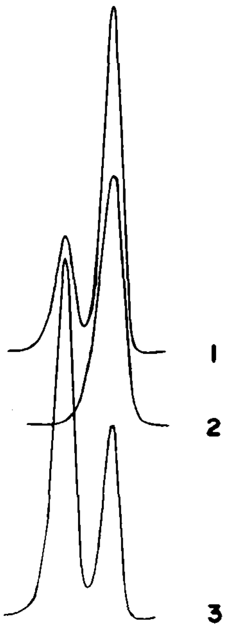


Figure 1—*Liquid chromatogram of (1) drug impurity, (2) 16α-methyl-17-ketone (IIa), and (3) epimerized 16α-methyl-17-ketone (II). Only that portion of the chromatogram showing 17-ketones is reproduced.*

hot stage by seeding directly on the material so as to favor the β-epimer.

The presence of the solid impurity was somewhat obscured by the dark glass container used to protect the injection solution from decomposition. The mechanical hazards from the presence of large amounts of an insoluble 17-ketone (II) in an injection solution are obvious. Possible adverse physiological activities, especially in larger doses, are obscure, because pharmacological studies on such impurities are limited. The situation is further complicated by the fact that the 16α- and 16β-methyl compounds (II) may show distinctly different activities.

Lodge *et al.* (9) reported that, of six manufacturers of dexamethasone for the Canadian market, five produced products containing up to 2% of the 17-ketone (II). These investigators found the same extent of contamination with the corresponding 17-oxo oxidation product in prednisone and prednisolone tablets on the Canadian market. Massive contamination (50%), as described in this report, and even lower levels could result in pronounced physiological activity caused by the presence of the 17-oxo contaminant, since patients on corticosteroid therapy may already be exposed to 0.4 mg of oxidation products daily. Some androgenic side effects of corticosteroids may be due to impurities (10).

Concerned that known unfavorable anabolic–androgenic ratios, which can occur from 17-keto or 17-hydroxy substitution, might be observed with dexamethasone, Lodge *et al.* (9) studied the anabolic and androgenic activities. They found that II had no demonstrable organ-stimulating effect; it caused a significant decrease in weight in the levator ani muscle but showed no inhibition of androgenic and anabolic activities. Their results (9) did indicate possible catabolic activity for this impurity; dexamethasone, particularly, is known to have catabolic effects (11). Drugs related to betamethasone may be expected to be subject to the same type of contamination from a 17-oxo product, consisting of, in this case, the 16β-methyl epimer (II*b*) (peak to the left in Fig. 1, curve 1). Alternatively, the same epimeric mixture obtained from the dexamethasone sodium phosphate sample would be expected when oxidation is accompanied by epimerization at the ketonic α-position.

The analysis method described here would apply in either case and possibly also in cases where cleavage occurred at a steroidal C_{17} position to generate a 17-ketone. The presence of an epimeric 17-oxo product as an impurity in dexamethasone sodium phosphate suggests that the oxidation occurred under acidic or basic conditions favorable to epimerization at the α-position of ketones. Dexamethasone itself is known to be highly sensitive to air oxidation; under basic conditions, 50% oxidation of the α-ketol side chain occurs within 6–8 min (12). This reaction and its mechanism have been studied extensively in the case of the kinetics of the base-catalyzed degradation of prednisolone (13).

In this study, the ease with which the dexamethasone degradation products could be determined by HPLC and the possible resolution provided by the reversed-phase column (14) simplified laboratory analysis. These advantages also gave a fast solution to problems encountered in the initial work by TLC, which did not separate the epimers and gave coincidental matching of R_f values with the R_f value of dexamethasone acetate during the first attempts to identify the impurity.

Significant interest has developed in the use of HPLC for analysis of epimers, *e.g.*, the determination of the 15-epimer of dinoprost in bulk drugs (15) and the assay for foreign epimeric steroidal impurities in methandrostenolone (16). In the latter case, one epimeric impurity exhibits an unfavorable anabolic–androgenic ratio; because of this serious problem, the presence of the impurity in any significant amount is undesirable and its detection is important. The use of HPLC for detection of epimers or various diastereoisomers responsible for specific undesirable physiological properties may become increasingly important in quality assurance programs.

A TLC method that distinguishes between the oxidation products was reported recently (17) (R_f values reported for the 16-methyl 17-ketones of dexamethasone and betamethasone were 0.60 and 0.59, respectively). A new quantitative TLC method, which is sensitive enough to allow determination of α to β ratios and which is useful for the analysis of large numbers of these 16-methyl-17-ketones in quality assurance programs, will shortly be reported by this laboratory.

REFERENCES

(1) "Infrared and Ultraviolet Spectra of Some Compounds of Pharmaceutical Interest," rev. ed., Association of Official Analytical Chemists, Washington, D.C., 1975, p. 128.

(2) J. J. Kirkland, "Modern Practice of Liquid Chromatography," Wiley, New York, N.Y., 1971, pp. 10, 14.

(3) R. Rausser and E. P. Oliveto, U.S. pat. 3,010,958 (1961); through *Chem. Abstr.*, **57,** 3524 (1962).

(4) M. Gut, U.S. pat. 3,529,598 (1970); through *Chem. Abstr.*, **74,** 54113 (1971).

(5) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 125.

(6) "Handbook of Analytical Toxicology," I. Sunshine, Ed., Chemical Rubber Co., Cleveland, Ohio, 1969, p. 428.

(7) E. M. Cohen, in "Analytical Profiles of Drug Substances," vol. 2, K. Florey, Ed., Academic, New York, N.Y., 1973, pp. 163–197.

(8) D. H. R. Barton and W. C. Taylor, *J. Chem. Soc.*, **1968,** 2500.

(9) B. A. Lodge, H. Watanabe, and L. Watts, *Can. J. Pharm. Sci.*, **10,** 107 (1975); through *Chem. Abstr.*, **84,** 26327 (1976).

(10) J. A. Vida, "Androgens and Anabolic Agents," Academic, New York, N.Y., 1969, pp. 77–91.

(11) H. E. B. Astwood and C. E. Cassidy, "Clinical Endocrinology," Greene & Stratton, New York, N.Y., 1968, p. 499.

(12) M. Pesez and J. Bartos, *Bull. Soc. Chim. Fr.*, **56,** 1928 (1962).

(13) D. E. Guttman and P. D. Meister, *J. Am. Pharm. Assoc., Sci. Ed.*, **47,** 773 (1958).

(14) "Manual No. CU 27386, Rev. B," Waters Associates, Milford, Mass., Sept. 1976.

(15) T. J. Roseman, S. S. Butler, and S. L. Douglas, *J. Pharm. Sci.*, **65,** 673 (1976).

(16) A. G. Butterfield, B. A. Lodge, N. J. Pound, and R. W. Sears, *ibid.*, **64,** 441 (1975).

(17) M. Lanquette and B. A. Lodge, *J. Chromatogr.*, **129,** 475 (1976).

ACKNOWLEDGMENTS

The authors thank Matthew L. Dow and William B. Furman for helpful suggestions, Paul Flinn for technical assistance, and John C. Black for assistance in the preparation of the figures.