Colorimetric Procedure for Determination of Methylprednisolone Hemi-ββ′-dimethylglutarate and Methylprednisolone in Aqueous Solutions

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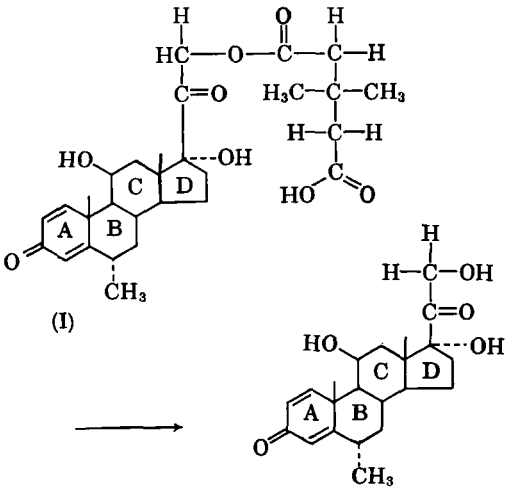
An analytical procedure for the determination of methylprednisolone hemi-ββ′-dimethylglutarate and its hydrolysis product, methylprednisolone, was developed. The method involves separation of the steroids by extraction, followed by their colorimetric assay.

ONE APPROACH to the production of an aqueous pharmaceutical preparation of a cortical steriod is the use of the hemiesters of dicarboxylic acids. Of particular interest is the hemiester of methylprednisolone[1] and ββ′-dimethylglutarate (DMG).

The hydrolysis of methylprednisolone DMG (I) yields methylprednisolone (II) which is also physiologically active. Upon degradation of the dihydroxyacetone side chain, methylprednisolone would yield one or more neutral and acidic compounds which absorb radiation in the ultraviolet region of the spectrum (1).



(I)

(II)

Although ring A is photolabile (2), this mode of degradation is not important in pharmaceutical preparations if suitable precautions are taken to provide for protection from light (3).

Thus, in the stability testing of an aqueous pharmaceutical preparation of methylprednisolone DMG, assay methods are required that would be specific for I and II in the presence of each other and in the presence of degradation products. Ultraviolet absorption spectroscopy is not applicable, even in the absence of extraneous ultraviolet absorbing materials, as both active species have the same ultraviolet chromophore, *i.e.*, the A ring. Partitioning between water at a high pH and an immiscible solvent followed by ultraviolet spectroscopy is not applicable, as the neutral and acidic degradation products would interfere when present (1).

An assay procedure was developed that separated the two active compounds by extracting an aqueous solution at a high pH with chloroform. The chloroform phase was assayed for methylprednisolone using the blue tetrazolium procedure (4, 5). The aqueous phase was assayed for methylprednisolone DMG by acidifying, extracting with chloroform, and applying the method of Porter and Silber (6). Neither of the methods is affected by the presence of degradation products (4, 7).

EXPERIMENTAL

Determination of Partition Coefficient vs. pH.—Aqueous solutions containing 500 mcg. of methylprednisolone DMG per ml. were prepared using 0.5 *M* phosphate buffers. Two-milliliter volumes of these solutions were equilibrated with 25 ml. of water-saturated chloroform at room temperature. The layers were separated and the amount of steroid in each phase was determined by ultraviolet spectroscopy. Hydrolysis of the ester was assumed to be negligible during the equilibration period. The results listed in Table I indicate that essentially all of the hemiester remains in the aqueous layer at pH 10.0.

Color Development of Methylprednisolone Hemi-ββ′-dimethylglutarate and the Porter-Silber Re-

TABLE I.—PARTITION COEFFICIENT FOR METHYLPREDNISOLONE ββ′-DIMETHYLGLUTARATE SODIUM BETWEEN WATER AND CHLOROFORM AT VARIOUS pH'S AT ROOM TEMPERATURE

pH	$C_{organic}/C_{aqueous}$
7.8	0.643
9.0	0.040
10.0	0.01

Received April 14, 1962, from Product Research and Development, The Upjohn Company, Kalamazoo, Mich.
Accepted for publication June 5, 1962.

[1] Marketed as Medrol by The Upjohn Company, Kalamazoo, Mich.

agent.—To our knowledge, the applicability of the Porter-Silber reagent to steroidal hemiesters such as the ββ′-dimethylglutarate has not been reported in the literature. Hence, the formation of color by methylprednisolone DMG in the Porter-Silber procedure was studied as a function of time, and compared to methylprednisolone.

Two-milliliter volumes of a 1.43×10^{-4} *M* 3A alcoholic solution of methylprednisolone DMG were mixed with 4-ml. volumes of a dilute sulfuric acid solution of phenylhydrazine hydrochloride (see Reagents, below), and with 4 ml. of the dilute acid only. In the same manner, 2-ml. volumes of 3A alcohol were mixed with the two reagents to serve as blanks. The reaction mixtures were heated for various times, 20–60 minutes, in a water bath at 60°, and then cooled rapidly in cold water. The absorbance was measured at 415 mμ in a 10-mm. cell with the appropriate 3A alcohol blank in the reference cell in each instance. The Porter-Silber color was determined by difference, *i.e.*, $A_{steroid} = A_{reagent\ sample} - A_{acid\ sample}$. A 1.43×10^{-4} *M* solution of methylprednisolone was handled in the same manner.

The data are presented graphically in Fig. 1 Although the color development with methylprednisolone DMG is not complete after 60 minutes at 60°, the rate of change is small enough, about 4%, that heating at 60° for 60 minutes would be satisfactory as an analytical procedure.

METHOD

Reagents.—*pH 10.0 buffer.* Seventy-one grams of anhydrous sodium dibasic phosphate, Mallinckrodt A.R. grade, was dissolved in 1000 ml. of water. The pH was adjusted to 10.0 with 1.0 *M* sodium hydroxide solution; *ca.* 30 ml. was required.

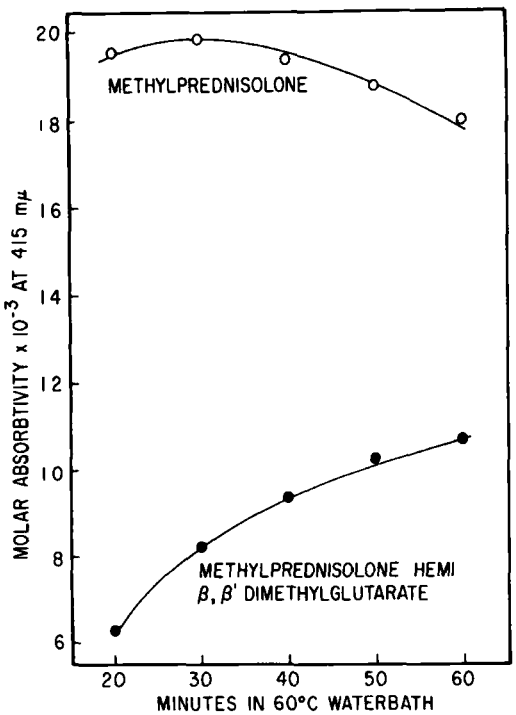


Fig. 1.—Development of the Porter-Silber color with methylprednisolone and with methylprednisolone hemi-ββ′-dimethylglutarate.

Blue tetrazolium solution. One hundred twenty-five milligrams of blue tetrazolium[2] was dissolved in 3A alcohol and diluted to 50 ml.

Tetramethylammonium hydroxide solution. Five milliliters of a 10% aqueous solution[3] was diluted to 50 ml. with 3A alcohol. These two solutions were usable for three days if refrigerated when not in use.

Sulfuric acid solution. Three hundred and ten milliliters of concentrated sulfuric acid and 190 ml. of water were mixed.

Phenylhydrazine hydrochloride solution. Phenylhydrazine hydrochloride[3] was recrystallized twice from 3A alcohol. One hundred fifty milligrams of the pure white material was dissolved in the dilute sulfuric acid and diluted to 100 ml. This reagent was prepared fresh daily.

Chloroform. Reagent grade.

Procedure.—Two-milliliter aliquots of aqueous samples containing 50–500 mcg./ml. of sodium methylprednisolone DMG and also of a standard solution were diluted with 3 ml. of pH 10 buffer and extracted with two 25-ml. portions of chloroform. The solvent was removed from the combined chloroform extracts under reduced pressure at room temperature. The dry residue was dissolved in 4 ml. of 3A alcohol. One milliliter of blue tetrazolium solution was added, followed by 0.5 ml. of tetramethylammonium hydroxide solution, and timing started. Forty-five ± 1 minutes later, the color development was quenched and the color was stabilized by adding 0.5 ml. of glacial acetic acid (8). The absorbance was measured at 525 mμ in a 10-mm. cell with 3A alcohol in the reference cell. A chloroform extract of 2 ml. of water treated in the same manner as the sample extract served as a reagent blank. Its absorbance was subtracted from that of the sample.

Beer's law was followed up to 12.5 mcg. methylprednisolone per ml. final volume.

The sample, after the removal of the methylprednisolone, and the water blank were acidified with 1 ml. of 6 *M* hydrochloric acid and extracted with 25 ml. of chloroform. A 10-ml. aliquot of the chloroform extract was taken to dryness. The residue was dissolved in 5 ml. of 3A alcohol. Two 2-ml. aliquots were transferred to glass-stoppered test tubes. To one of the aliquots was added 4 ml. of the phenylhydrazine solution; to the other was added 4 ml. of the dilute sulfuric acid. Both were heated for 60 minutes in a water bath at 60° and then cooled rapidly in cold water. The absorbance was measured at 415 mμ with the appropriate blank in the reference cell. The absorbance, due to the steroid, was obtained by difference, *i.e.*, $A_{steroid} = A_{reagent\ sample} - A_{acid\ sample}$.

The molar absorptivity for methylprednisolone ββ′-dimethylglutarate sodium was found to be approximately 10,000. Beer's law followed up to 53 mcg. per ml. final volume.

DISCUSSION

The results of the partition study indicate that a small amount of hemiester accompanies the extrac-

[2] Certified reagent marketed by Fisher Scientific Co.
[3] Marketed by Eastman Organic Chemicals.

tion of methylprednisolone. The molar absorptivity of the hemiester in the blue tetrazolium procedure is 513, while that of the steroid alcohol is 29,190. Therefore, the effect of minor amounts of the hemiester on the assay of methylprednisolone would be negligible.

From Fig. 1 it is seen that on a molar basis methylprednisolone DMG is 61% as chromogenic as methylprednisolone. It is interesting to note that the chromogenicities of hydrocortisone acetate and cyclopentylpropionate in the Porter-Silber reaction are equal to that of hydrocortisone (9).

Marcus (10) determined hydrocortisone phosphate with the Porter-Silber procedure by heating for 2 hours at 60°. He did not state the chromogenicity of the phosphate ester relative to hydrocortisone under the same conditions.

The sample of steroid ester used in this investigation was found by papergram analysis to contain approximately 2% of the steroid alcohol. The necessity of purifying the material was circumvented by a synthetic sample approach. Known amounts of methylprednisolone were added to the chloroform used to extract a standard volume of hemiester solution, and the samples were assayed using the procedure described. The results of these assays are listed in Table II. A plot of micrograms of methylprednisolone added *vs.* micrograms found yielded a straight line, as shown in Fig. 2. The intercept of the line indicates that 2.63 ± 0.04% steroid alcohol was present in the sample of hemiester.

The average variation of individual results from the average of duplicate results was 2.5% for the methylprednisolone DMG in simple systems. The standard deviation for the methylprednisolone assay was ±1.3%.

The procedure was applied successfully to pharmaceutical formulations.

TABLE II.—THE ADDITION OF KNOWN AMOUNTS OF METHYLPREDNISOLONE TO METHYLPREDNISOLONE ββ′-DIMETHYLGLUTARATE KNOWN TO CONTAIN SOME METHYLPREDNISOLONE[a]

Methylprednisolone, mcg.	
Added	Found
0	26.0, 26.1, 27.6, 27.6
10.0	33.7, 35.3, 36.9, 37.6
25.0	49.7, 49.9, 50.0, 52.2
40.0	65.5, 66.1, 67.2, 67.6, 68.0
50.0	76.0, 76.2, 76.4, 76.7, 76.8

[a] Slope of the least squares line = 1.001 ± 0.013 (S.D.). Intercept 26.26 ± 0.42. Since 1000 mcg. of hemiester was taken, % of methylprednisolone present = 2.63 ± 0.04.

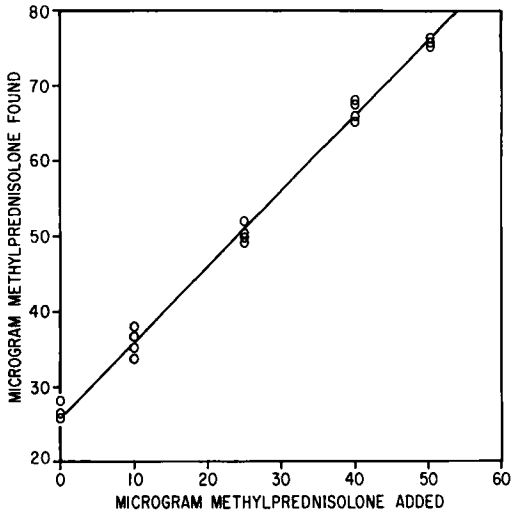


Fig. 2.—The addition of known amounts of methylprednisolone to methylprednisolone ββ′-dimethylglutarate known to contain some methylprednisolone.

SUMMARY AND CONCLUSIONS

A colorimetric method is described for determining methylprednisolone ββ′-dimethylglutarate and methylprednisolone in aqueous solution in the presence of each other and in the presence of degradation products.

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