same maximum activation and emission wavelengths as described for 2′-aminoprobenecid (13).

DISCUSSION

The basis of the fluorescence method is conversion of probenecid to an anthranilic acid derivative. Blanchard *et al.* (13) reduced 2′-nitroprobenecid to 2′-aminoprobenecid and described the fluorescent properties of the latter. This observation was applied to the assay of probenecid by first nitrating the drug and then reducing the nitro group. However, the resulting fluorescent product was not 2′-aminoprobenecid, a relatively nonpolar compound that could be extracted into organic solvents, but the more polar *p*-sulfoanthranilic acid. Consequently, interfering substances such as salts and degradation products of biological origin (blank) were only poorly separated from the fluorescent probenecid derivative. The present method achieves a partial separation of interfering substances by means of a charcoal adsorption–elution step. A more effective separation to maximize fluorescence, such as TLC or high-pressure liquid chromatography, seems indicated.

While the fluorometric method for probenecid has only severalfold greater sensitivity than the spectrophotometric method for assay in cerebrospinal fluid and less sensitivity for plasma, there is no significant interference by drugs such as salicylate, unlike the interference by this compound in the UV assay (14). Since metabolites of probenecid are poorly extracted by ethylene dichloride (16), the method is generally specific for probenecid.

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Determination of Fluoxymesterone, Norethandrolone, Prednisolone, and Prednisone in Tablets by Differential Pulse Polarography

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Received February 1, 1977, from the *Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada, M5S 1A1.* Accepted for publication June 22, 1977.

Abstract □ A differential pulse polarographic method for the determination of fluoxymesterone, norethandrolone, prednisolone, and prednisone in tablets is described. This method is more sensitive than dc polarography, and the measurement of diffusion current is greatly simplified. Sørensen phosphate buffer, pH 5.6, was used as the supporting electrolyte. No apparent interference was observed from tablet excipients; the method is rapid, simple, and relatively precise.

Keyphrases □ Fluoxymesterone—differential pulse polarographic analysis in tablets □ Norethandrolone—differential pulse polarographic analysis in tablets □ Prednisolone—differential pulse polarographic analysis in tablets □ Prednisone—differential pulse polarographic analysis in tablets □ Differential pulse polarography—analyses, fluoxymesterone, norethandrolone, prednisolone, and prednisone in tablets □ Androgens—fluoxymesterone and norethandrolone, differential pulse polarographic analysis in tablets □ Glucocorticoids—prednisolone and prednisone, differential pulse polarographic analysis in tablets □ Steroids—fluoxymesterone, norethandrolone, prednisolone, and prednisone, differential pulse polarographic analyses in tablets

The differential pulse polarographic assay of several Δ^4-3-ketosteroids in parenteral formulations (1) and oral contraceptive tablets (2) recently was reported. In addition, the polarographic reduction of certain other Δ^4-3-ketosteroids is well documented (3–7), and a number of workers reported the dc polarographic assay of steroids having this same α,β-unsaturated keto group in pharmaceutical preparations (8–10). Several reports concerned the application of colorimetric (11, 12), spectrophotometric (13, 14), and titrimetric (15) procedures for the estimation of prednisone, prednisolone, and other corticosteroids in pharmaceutical dosage forms.

This paper reports a simple and rapid differential pulse polarographic method for the analysis of fluoxymesterone (I), norethandrolone (II), prednisolone (III), and prednisone (IV) in commercial tablets.

EXPERIMENTAL

Polarographic Analysis—Assays were performed using a polarographic analyzer[1] equipped with a drop timer[2] in the differential pulse

[1] Princeton Applied Research model 174A.
[2] Princeton Applied Research model 172A.

Table I—Current Peak Height *versus* Steroid Concentration

Steroid	E_p, v (*versus* SCE)	Concentration, μg/ml				
		5	10	20	30	40
		Relative Peak Height				
I	−1.44	4.7	8.9	17.0	25.2	32.8
II	−1.35	10.1	21.4	42.4	57.4	68.9
III	−1.19	7.0	15.0	32.7	44.5	53.7
IV	−1.16	10.6	20.8	37.7	50.9	58.9

mode. A three-electrode polarographic cell containing a dropping mercury electrode (DME) as the analyzer electrode, a saturated calomel electrode (SCE) as the reference electrode, and platinum wire as an auxiliary electrode was used.

The dropping mercury electrode had a drop time of 2 sec and a flow rate of 1.5 mg/sec. The capillary characteristic ($m^{2/3}t^{1/6}$) was 1.471 measured in Sørensen phosphate buffer (16) with an open circuit and at a mercury column height of 95 cm. The current range was either 2 or 5 μamp. Other parameters were: pulse amplitude, 25 mv; and scan rate, 2 mv/sec. The potential range scan in volts for each steroid was: I, from −1.15 to −1.90; II, from −1.11 to −1.86; III, from −1.0 to −1.75; and IV, from −0.95 to −1.70. All polarograms were recorded on an *x–y* recorder[3].

A pH meter[4] fitted with a combination glass–saturated colomel electrode pair was used to measure the pH of all solutions.

Reagents and Chemicals—All chemicals were analytical reagent grade. The pure steroids, obtained from the manufacturers, were used without further purification. The purity of each steroid reported by the manufacturer was: fluoxymesterone[5], 98.5%; norethandrolone[6], 99.26%; prednisolone[7], 99.37%; and prednisone[8], 101.1%.

Calibration Curve—A stock solution was prepared for each steroid by dissolving 20 mg in 100 ml of solvent. Ethanol (95%) was the solvent used for I and IV, while methanol was used for II and III. Suitable aliquots of each stock solution were transferred to separate 25-ml volumetric flasks, and a sufficient quantity of the appropriate organic solvent (ethanol or methanol) was added to produce a volume of 5 ml before the flask was filled to the mark with pH 5.6 Sørensen phosphate buffer.

Triplicate polarograms were recorded for each standard solution in the series, which covered the 5–40-μg/ml range. Aliquots of 20 ml of these solutions were transferred to the cell in turn and deoxygenated with pure nitrogen for 5 min prior to obtaining the polarograms in the quiescent solution while a nitrogen layer was maintained over the solution surface. Polarograms were obtained using the differential pulse mode. Figure 1 shows the actual polarograms of one standard solution of each steroid employed.

Assay of Tablets—Twenty tablets were weighed and finely powdered.

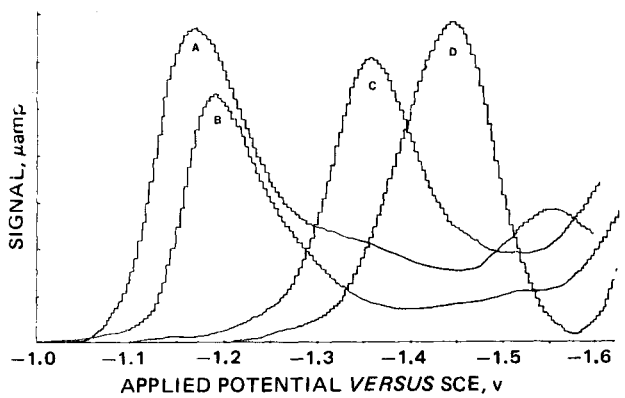

Figure 1—*Differential pulse polarograms for prednisone (A), prednisolone (B), norethandrolone (C), and fluoxymesterone (D). The concentrations of the steroids used were 10, 30, 40, and 50 μg/ml with sensitivities of 0.2, 0.2, 0.5, and 0.5 μamp (full-scale deflection) for C, D, B, and A, respectively.*

[3] Houston Omnigraphic model 2000.
[4] Fisher Accumet model 230 pH/ion meter.
[5] Fluoxymesterone USP, lot R-4185, The Upjohn Co. of Canada Ltd., Don Mills, Ontario, Canada.
[6] Norethandrolone, lot V-183, G. D. Searle & Co., Chicago, Ill.
[7] Prednisolone (anhydrous) USP, batch 508BT, Novopharm Ltd., Scarborough, Ontario, Canada.
[8] Prednisone USP, batch 977ED, Novopharm Ltd., Scarborough, Ontario, Canada.

Table II—Assay Results of Commercial Steroid Tablets

Steroid and Labeled Amount per Tablet	Percent of Labeled Amount Found	
	Polarographic[a] Assay	Manufacturer's Assay
I, 5 mg	92.7 ± 1.6	96.0[b]
II, 10 mg	101.8 ± 0.85	100.3[c]
III, 5 mg	103.7 ± 0.66	103.4[d]
IV, 5 mg	104.7 ± 1.2	103.8[d]

[a] Mean ± %*SD* of five separate determinations with duplicate measurements on each sample solution. [b] High-pressure liquid chromatography, USP XIX. [c] UV spectrophotometric method, NF XIII. [d] Visible spectrophotometric steroid assay, USP XIX.

An amount of tablet powder equivalent to about 7 mg of steroid was accurately weighed and transferred, with the aid of about 50 ml of the appropriate solvent, to a 100-ml volumetric flask. This quantity of steroid should ensure that the peak height value will correspond to the linear portion of the calibration curves plotted from the data in Table I. Then the flask was shaken mechanically[9] for 20 min and diluted to volume with the same solvent.

The flask contents were mixed and excipients were allowed to settle. A 5.0-ml aliquot was pipetted into a 25-ml volumetric flask and diluted to volume with pH 5.6 Sørensen phosphate buffer as the supporting electrolyte. A 20-ml aliquot of this solution was transferred to the polarographic cell. The remainder of the procedure was identical to that described for the calibration curve.

RESULTS AND DISCUSSION

Several investigators reported (17–19) that only those compounds in which the keto group is conjugated with a double bond give rise to a polarographic wave. Kabasakalian and McGlotten (20) studied polarographic reduction of prednisone and prednisolone in well-buffered 50% ethanol solutions and discussed the effects of pH, temperature, concentration, and mercury column height on the polarographic wave. Mixtures of prednisone ($E_{1/2}$ = −1.2 v) and cortisone ($E_{1/2}$ = −1.36 v) can be analyzed with an accuracy within 4% in a 50% methanol solution buffered at pH 5.5 with sodium acetate–acetic acid (21).

Preliminary experiments revealed that all four steroids (I–IV) exhibited well-defined peaks when pH 5.6 Sørensen phosphate buffer was used as a supporting electrolyte (Fig. 1). The peak potentials, E_p, for these steroids are listed in Table I.

The calculation of diffusion current for quantitation is greatly simplified by employing differential pulse polarography rather than dc polarography. The display of the signal in a Gaussian-shaped curve can be measured easily without time-consuming extrapolations for limiting and residual currents as required in classical dc polarography. The instrumental artifacts in differential pulse polarography[10] were discussed by Christie *et al.* (22).

The position of the peak is independent of concentration, and the peak heights are linearly dependent on concentration over the 5–20-μg/ml range for these steroids, with a negative deviation at 30 and 40 μg/ml. Classical dc polarography cannot attain sufficient sensitivity to measure these concentration levels. Table I gives the peak height values for the different steroid concentrations studied. These data were plotted, and the calibration curves were used to determine the steroid concentrations in the sample solutions of the corresponding steroids.

Results obtained by applying the proposed polarographic procedure to the commercially available tablets are presented in Table II. Comparison of the assay results with those obtained by the manufacturers shows a good correlation. The accuracy of the proposed method is evident from the data. The final polarographic assay results for tablets were corrected to account for the percent purity in the reference steroid, using the manufacturer's data for each steroid.

The USP (23) assay of prednisone and prednisolone tablets by the blue tetrazolium procedure is time consuming. Therefore, automated procedures were suggested (24, 25) for single-tablet assays when performing USP content uniformity tests. The procedure involves extraction with chloroform. Aliquots of chloroform are then evaporated, and steroids are redissolved in alcohol. Color is developed by the reduction of blue tetrazolium. The differential pulse polarographic procedure described here is applied directly to the sample extract without additional manipulation

[9] Wrist-action shaker, Burrell Corp., Pittsburgh, Pa.
[10] Princeton Applied Research model 174 polarographic analyzer.

except for dilutions. This procedure is much more convenient than extracting the steroid with a solvent that must be removed before determination. The insoluble constituents in these tablets do not cause any interference and need not be removed before differential pulse polarography. The drug is quantitatively dissolved in the solvent upon shaking for 20 min.

This differential pulse polarographic procedure offers the advantages of sensitivity, speed of analysis, and moderate cost of apparatus. Since the samples are analyzed without prior separation, the procedure results in a considerable saving of time, particularly when a large number of samples are assayed or when a large number of single tablets must be assayed to establish content uniformity.

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The authors are indebted to the pharmaceutical companies for their generous contributions of reference standards and dosage forms.

COMMUNICATIONS

Assay of Amygdalin Dosage Forms from Mexico

Keyphrases □ Amygdalin dosage forms—IR and NMR spectral identification and high-pressure liquid chromatographic analysis of components in various products □ IR spectroscopy—identification, components in various amygdalin dosage forms □ NMR spectroscopy—identification, components in various amygdalin dosage forms □ High-pressure liquid chromatography—analyses, amygdalin and components in various dosage forms

To the Editor:

One controversial topic in cancer treatment today is whether laetrile (1) (amygdalin[1]) is effective as a cancer chemotherapeutic agent. To resolve this complicated issue, the National Cancer Institute (NCI) considered a clinical trial to test the efficacy of amygdalin in cancer treatment. "Available" amygdalin dosage forms were procured and evaluated to determine their suitability for such a trial. Although the question of efficacy in humans may not be answered for a long time, the chemical compositions of the title amygdalin formulations are now known. Because this information has not been previously available to the public[2], it should be of vital interest to current and future users of these dosage forms. For this reason, we are reporting our findings in this communication.

Most amygdalin formulations used by cancer patients in this country are produced in Mexico, and samplings of the products we assayed originated from that country. Our samplings were selected randomly from groupings of these formulations released to NCI by U.S. Customs, which had seized the amygdalin products as they were being transported illegally into the United States. The seized materials consisted of five groups[3] of injectable liquids in amber ampuls and three groups[3] of tablets for oral administration. The amber ampuls had a 10-ml fill volume capacity. A ceramic label indicated the contents as "Amigdalina 3 g." and identified the supplier as Cyto Pharma de Mexico, S.A. The label also stated that the product was an injec-

[1] The trivial names amygdalin, laetrile, nitriloside, vitamin B-17, and 1-mandelonitrile-β-diglycoside have been used synonymously by both the proponents and opponents of laetrile (2). Systematically, laetrile is (*R*)-mandelonitrile-β-glucuronic acid (I) and amygdalin is (*R*)-mandelonitrile-β-D-glucosido-6-β-D-glucoside (II). The drug currently offered as an antitumor agent is amygdalin, which is usually extracted from kernels of apricots and related fruits.

[2] Levi *et al.* (3) described briefly the physicochemical and biochemical properties of two pharmaceutical formulations of amygdalin available in the United States and Canada in the early 1960's. The authors identified neither the manufacturers nor the dosage potencies. However, a comparison of the reported data with those in this article indicates that the earlier products differed from those available today.

[3] According to U.S. Customs records, these groups were seized between 1973 and 1975; all groups, except Group 76-2505-11569, contained at least 1000 dosage units. The seized materials were kept in an air-conditioned vault from the time of seizure to the time of release, July 1977.