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Note

Description and chromatographic investigation of Mexican medication for arthritis and asthma, including an unusual corticosteroid

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Tablets and capsules from Mexican sources are frequently sent to these laboratories for identification and analysis. The recent report by Byrne *et al.*[1] prompts us to record our findings, which include the identification of a non-compendial corticosteroid. Product recognition codes and simple chromatographic procedures for the separation and identification of the component drugs are given.

EXPERIMENTAL

Materials

16α-Methylprednisone acetate and 16β-methylprednisone acetate were prepared from the corresponding methylprednisone (kindly donated by Schering, Berlin, G.F.R.) by reaction with acetic anhydride in pyridine[2]. Authentic samples of the other drugs were obtained from the Branch's reference collection. Thin-layer chromatography (TLC) (Tables I and II) was performed on 0.25-mm silica gel layers using precoated 20 × 20 cm glass plates (silica gel G-F 254, Brinkmann, Westbury, N.Y.,

TABLE I

THIN-LAYER CHROMATOGRAPHY OF DRUGS —R_F VALUES*

A, Chloroform saturated with ammonia–methanol (18:1); B, acetone–12 *N* ammonia (99:1); C, ethanol–5 *N* ammonia (9:1); D, methylene chloride–dioxane–water (100:50:50); use lower (filtered) layer; E, ethylene chloride–methanol–water (95:5:0.2).

Drug	*System*				
	A	*B*	*C*	*D*	*E*
Triamcinolone	0.06	0.75	0.58	0.40	0.05
16β-Methylprednisone acetate	0.77	0.87	0.74	0.93	0.38
Diazepam	0.90	0.78	0.72	0.95	0.65
Chlorpheniramine	0.82/0.00	0.80/0.33	0.42/0.10	0.07	0.05
Estradiol 17-valerate	0.77	0.88	0.76	1.00	0.63
Meprobamate	0.28	0.78	0.68	0.63	0.15
Indomethacin	0.00	0.88	0.65	0.92	0.20

* Two spots developed with chlorpheniramine maleate in systems A, B and C.

TABLE II

THIN-LAYER CHROMATOGRAPHY OF CORTICOSTEROIDS —RELATIVE R_F VALUES

16β-Methylprednisone acetate $R_F = 1.00$; see Table I for systems D and E.

Corticosteroid	*System*	
	D	*E*
Betamethasone	0.66	0.26
Cortisone acetate	1.04	1.21
Dexamethasone	0.66	0.24
Fludrocortisone acetate	1.01	0.94
Fluperolone acetate	1.01	1.00
Hydrocortisone	0.62	0.24
Hydrocortisone acetate	0.97	0.85
Paramethasone acetate	1.03	1.12
Prednisolone	0.51	0.19
Prednisolone acetate	0.88	0.72
Prednisone	0.73	0.47
Prednisone acetate	1.01	1.03
Triamcinolone	0.40	0.12
Triamcinolone diacetate	0.88	1.00
Beclomethasone dipropionate		1.62
Beclomethasone 17-propionate		0.82
Beclomethasone 21-propionate		1.16
Betamethasone 17-valerate		0.85
Deoxycortone acetate		1.85
Deoxycortone pivalate		1.94
Methylprednisolone		0.19
Methylprednisolone acetate		0.78
Prednisolone acetate		0.72
Triamcinolone acetonide		0.65

U.S.A.). The developing solvents are given in Table I. Spots were detected by the quenching of ultraviolet (UV) fluorescence and by placing the plate in a tank of iodine vapours. A 50% ethanolic sulfuric acid spray was also used in the detection of the steroids, and colour comparisons were made before and after heating in an oven at 120° for 5 min. Gas–liquid chromatography (GLC) was performed on a Hydro-Flow Series 3000 instrument, employing 3% OV-17 on a support of Chromosorb W HP (80–100 mesh; Chromatographic Specialties, Brockville, Canada) packed in a 6 ft. × 0.15 in. I.D. glass column, carrier gas (nitrogen) at 30 ml/min, oven temperature 250°, injector and detector temperatures 275° (see Table III). Infrared (IR) spectra were

TABLE III

GAS CHROMATOGRAPHY OF DRUGS —RETENTION TIMES*

Drug	*Retention time (min)*
Diazepam	10.5
Chlorpheniramine	2.3
Estradiol 17-valerate	40.8
Indomethacin	13.7

* The nature of the emergent material was not determined.

determined using KBr disks on a Perkin-Elmer 621 spectrophotometer. UV spectra (Table IV) were recorded in methanolic or ethanolic solutions with a Beckman DBGT spectrophotometer. Proton magnetic resonance (PMR) spectra of solutions in $CDCl_3$ were determined on a Varian A-60A instrument.

TABLE IV

UV ABSORPTION MAXIMA

Drug	*Wavelength maximum (nm)*
Triamcinolone	237
16β-Methylprednisone acetate	236
Diazepam	230
Chlorpheniramine	261*
Estradiol 17-valerate	280**
Indomethacin	231

* Secondary maxima at 257 and 266 nm.
** Shifted to 288 and 297 nm on addition of alkali.

Procedures

Preparations were measured with a micrometer, weighed and coded[3,4] (Tables V and VI). One powdered tablet or the contents of one capsule were placed in a 20-ml screw-capped vial or centrifuge tube. The caps employed had PTFE linings from which the backing glue had been removed by previous extraction with chloroform. Ethyl acetate (10 ml) was added. The mixture was rotated (Multi-Purpose Rotator, Scientific Industries, Springfield, Mass., U.S.A.) at 20 rpm for 1 h. The mixture was then centrifuged or filtered and the clear supernatant was evaporated to dryness in a tared tube by warming it under a gentle stream of nitrogen. Rigorous identification was made by recording a KBr disk IR spectrum of the residue and comparing it with standard spectra. A portion of the residue was redissolved in ethyl acetate or methanol for TLC and GLC examination. A second tablet or capsule, or a portion of the previous residue was extracted into methanol or ethanol and a UV spectrum was recorded in quartz cells against a solvent blank and compared with standard spectra.

RESULTS AND DISCUSSION

Three different tablet samples contained the same component which did not correspond in R_F or in IR characteristics with the standard drugs initially employed. Each of these tablets was friable, weighed 175 mg and measured 8.1 × 2.8 mm (Table V). The presence of a corticosteroid was suspected from three factors: (i) TLC behaviour and development of a brownish spot on spraying the chromatogram with sulfuric acid; (ii) UV maximum at 236 nm and (iii) medical indications. The IR spectrum showed four bands in the carbonyl stretching region (1650–1750 cm^{-1}) suggesting that at least four carbonyl groups were present, one probably being an acetate (1750 cm^{-1}) since there was also a strong band at 1240 cm^{-1}. The PMR spectrum generally resembled those of corticosteroids and showed doublets (J = 10 Hz) at 6.20 (showing further fine splitting) and 7.60 ppm, and a broad singlet at 6.12 ppm. These chemical shifts closely resembled those of the olefinic protons of both predni-

TABLE V

DIGIT CODE FOR THE IDENTIFICATION OF DRUG SAMPLES

For index to the coding, see Table VI.

Tablet appearance	*Coating*	*Top view*	*Side view*	*Colour type*	*Outside colour*	*Marking*	*Inside colours*	*Inside colour*	*Scoring*	*Size (mm)* *	*Weight (mg)* *	*Identification (quantity)* *
Small white	1 1	3	2	1	1	1	1	1	1	6.4 × 2.8	110	Triamcinolone (3 mg)
Small, light orange	1 1	3	2	1	6**	1	1	6**	1	6.5 × 2.8	110	Triamcinolone (3 mg)
White	1 1	3	3	1	1	1	1	1	2	8.1 × 2.8	175	16β-Methylprednisone acetate (4 mg)
Salmon	1 1	3	3	1	6	3***	1	6	2	8.0 × 2.4	165	Diazepam (4 mg)
Light green	1 1	3	3	1	8	3***	1	8	2	8.0 × 2.4	165	Diazepam (2 mg)
Mottled green	1 1	3	3	3	8	1	1	8	2	8.1 × 2.8	175	16β-Methylprednisone acetate (4 mg)
Speckled grey	1 1	3	3	4	2	1	2	2	2	8.1 × 2.8	175	16β-Methylprednisone acetate (4 mg)
Red, oval	1 3	2	2	1	5	1	1	1	1	9.2 × 6.7 × 4.7	270	Chlorpheniramine (6 mg)§
Orange	1 3	3	2	1	6	1	1	1	1	9.2 × 5.8	400	Chlorpheniramine (11 mg)§
Small, blue	1 3	3	2	1	9	1	1	1	1	6.9 × 4.0	140	Estradiol 17-valerate (2 mg)
Large, rose	1 3	4	2	1	5	2§§	1	1	1	17.5 × 8.7 × 6.1	850	Meprobamate (400 mg) Conjugated estrogens(0.4mg)
Large, green	1 3	4	2	1	8	2§§	1	1	1	14.5 × 7.4 × 5.3	500	Meprobamate (200 mg) Conjugated estrogens(0.4mg)
Yellow No. 2 capsule	3 2	1	1	1	7	1	1	1	4	18.6 × 6.1	320	Indomethacin (25 mg)

* Approximate.
** Very pale.
*** MJ.
§ Calculated as equivalent to the maleate.
§§ Ayerst; name may be rubbed off tablet.

TABLE VI

INDEX TO THE CODING OF TABLETS (T) AND CAPSULES (C)
For code, see Table V.

Column			
1		Tablets:	1 = tablets
1		Capsules:	2 = soft gelatin, 3 = hard gelatin
2	T	Coating:	1 = uncoated, 3 = sugar
2	C	Contents:	2 = powder
3	T	Top view:	2 = elliptical, 3 = circular, 4 = oblong
3	C	Shape:	1 = conventional
4	T	Side view:	2 = biconvex, 3 = flat with bevel
4	C	Transparency:	1 = opaque, 2 = clear
5	T	Colour type:	1 = solid, 3 = mottled, 4 = speckled
5	C	Capsule colour:	1 = single
6	T and C	Outside colour:	1 = white, 2 = grey, 5 = red, 6 = orange, 7 = yellow, 8 = green, 9 = blue
7	T and C	Marking:	1 = unmarked, 2 = manufacturer's name, 3 = initials
8	T and C	Inside colours:	1 = single, 2 = multiple
9	T and C	Inside colour:	1 = white, 2 = grey, 6 = orange, 8 = green
10	T	Scoring:	1 = unscored, 2 = half scored
10	C	Size:	2 = No. 4 capsule, 4 = No. 2 capsule

sone acetate and androsta-1,4-diene-3,11,17-trione (Δ^1-adrenosterone), which have an 11-oxo function. The presence of a secondary methyl group was inferred from the doublet (J = 7 Hz) at 1.13 ppm. An acetate was suggested by the sharp singlet at 2.17 ppm. It was deduced that the structure of the substance comprised a prednisone skeleton probably acetylated at position 21 and carrying a secondary methyl group probably at position 6 or 16. Both 16α- and 16β-methylprednisone acetate were prepared and their IR and PMR spectra were compared with those of the extract. The spectra of the unknown and also its TLC behaviour were identical with those of 16β-methylprednisone acetate, which concluded the identification.

The other drugs described in Table V were rigorously identified by IR spectrophotometry of the extracts. TLC investigations showed that the substances could be distinguished from one another using two systems (B and E, Table I). Further work with representative steroids indicated difficulties in distinguishing between 16β-methylprednisone acetate, fluperolone acetate and prednisone acetate by TLC alone (Table II). Triamcinolone, meprobamate and 16β-methylprednisone acetate appeared to undergo decomposition during GLC procedures, but the other drugs identified were easily distinguishable (Table III). The UV spectra of these substances (Table IV) show the expected similarity between 16β-methylprednisone acetate and triamcinolone which have the same ring A chromophore. The UV absorption was used to estimate the drug strengths indicated in the identification codes[3,4] (Tables V and VI). In the case of meprobamate which has a very weak UV absorption, quantitation was obtained by simple weighing of the extract; conjugated estrogens were shown to be present in these formulations by GLC[5].

CONCLUSIONS

Medications obtained from Mexico for the treatment of arthritis and asthma include chlorpheniramine, diazepam, meprobamate, estradiol 17-valerate, triamcinolone and 16β-methylprednisone acetate. The last is not marketed in the United States or Canada. Tentative identification can be made using product recognition codes and TLC.

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