TABLE I—APPARENT $E_{1/2}$ VALUES OF Pb(II)

Buffer	pH	$-E_{1/2}$[a]
Ascorbate	2.5	0.39
	3.7	0.41
	4.2	0.42
	4.8	0.43
Ternary	4.5	0.47
	6.5	0.48
	8.0	0.49
	8.9	0.52
	11.0	0.58

[a] With reference to the saturated calomel electrode.

ascorbate buffer a nearly constant shift is observed and the slope of the straight lines is about 0.08_2 v./pH. In the ternary buffer a nearly identical slope (0.08_5 v./pH) was found for the antimonyl ion. In presence of antipyrine however three breaks were noticed, the slope of the middle part ($5 \leqslant$ pH $\leqslant 9.5$) being about 0.11_2 v./pH. Aminopyrine showed a similar effect on the polarographic behavior of the antimonyl ion.

With regard to Pb(II) the apparent $E_{1/2}$ values are indicated in Table I. Generally for lead a rather slight shift of the half-wave potentials is observed as a function of pH. The pyrazolones show a practically negligible shift of the $E_{1/2}$ values.

The As(III) behaves in an entirely different way: the reduction waves only originate sufficiently with a pH $\leqslant 2.5$. Beyond this limit value they are badly shaped or even absent. Adding antipyrine causes distortion of the waves. The same effect occurs with aminopyrine.

Polarograms registered in the alkaline range show beside the accustomed cathodical waves polarographic currents emerging from potential zero. It concerns apparently anodic waves. These anodic waves have been shown to be generated for Sb and As in a strongly alkaline medium (0.1 to 1 *M* in KOH) by Kolthoff and Probst (2). These waves begin to appear at about pH 9. A shift of the $E_{1/2}$ values seems also to appear when the pH values increase.

CONCLUSION

Apparently the antimonyl ion distinguishes itself from Pb(II) and As(III) by the values of the half-wave potentials in the described buffers and furthermore also by the interaction with pyrazolone derivatives. Only the antimonyl ion shows a definite tendency to complex formation.

For the As(III) the occurrence of waves is limited to a determined pH range; the waves are distorted in the presence of the pyrazolones. The described data will be further developed with regard to possible analytical applications.

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Keyphrases

Pyrazolone derivatives
Antimonyl ion—pyrazolone derivative interaction
Buffer system—"ternary"
Polarographic study—complex formation

Stability of Prednisolone in an Organic Vehicle

By W. H. BOWLES

Prednisolone (0.7042 Gm.) was dissolved in 35 ml. of a vehicle (CMP) composed of 50 percent camphor, 25 percent *m*-cresyl acetate, and 25 percent *p*-chlorophenol, a mixture used in dentistry as a pulp-capping agent to reduce sensitivity in dental restorations. This preparation was assayed by the blue tetrazolium reaction and compared with an identically treated sample of USP reference standard prednisolone. The prednisolone–CMP mixture was then incubated at 60° with aliquots collected for assay at intervals of 24 hr., 5 days, 1 week, 2 weeks, and 6 weeks. At the end of 2 weeks of incubation, 99.3 percent of the prednisolone still remained; after 6 weeks' incubation, the prednisolone concentration had dropped to 72.4 percent of the preincubation concentration. Compared to literature values on the thermal stability of aqueous preparations of prednisolone, these results indicate that prednisolone in CMP is quite stable.

THE WIDESPREAD use of natural and synthetic steroids as anti-inflammatory agents in recent years has led to some concern as to the stability of these compounds in various preparations. Most of these preparations are suspensions of the steroid in aqueous or buffered aqueous media. Granulation procedures used in the manufacture of such preparations often involve exposure of the steroid to moisture and elevated temperatures (1).

Studies of prednisolone in aqueous suspensions of solid buffering agents, such as magnesium oxide, magnesium trisilicate, aluminum hydroxide, and calcium carbonate, have shown that agents which

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increase the pH of the suspension, *e.g.*, magnesium oxide, cause rapid deterioration of the prednisolone when incubated at 37.5 (1). Guttman and Meister (2) showed that the rate of degradation of prednisolone in aqueous alkaline suspensions was dependent upon the concentration of alkali. In this study, the rate of anaerobic degradation was only slightly below the rate of aerobic degradation.

In a study of the effect of the concentration of buffers on the rate of prednisolone breakdown, Oesterling and Guttman (3) found that the rate of degradation increased as the concentration of buffer increased. It was also shown that addition of a sequestering agent (EDTA-Na_2) could greatly decrease the rate of degradation of prednisolone. In this same series of studies, when the temperature was increased from 35° to 70° the rate of degradation was increased approximately sevenfold at pH 8.

The present study is concerned with the stability of prednisolone in an organic medium (CMP), composed of 50% camphor, 25% *m*-cresyl acetate, and 25% *p*-chlorophenol. This combination, with prednisolone in 1% concentration, has been used as a pulp-capping agent to reduce thermal sensitivity in dental restorations since about 1960 (4, 5). Mosteller (5, 6) has expressed doubt as to the stability of the combination beyond a period of 90 days. However, apparently no studies have been done on the stability of prednisolone in organic vehicles and in view of the relative instability of prednisolone in aqueous and buffered suspensions, it was deemed worthwhile to examine the stability of prednisolone in the camphor–*m*-cresyl acetate–*p*-chlorophenol (CMP) mixture.

METHODS AND MATERIALS

Prednisolone (0.7042 Gm.) was dissolved in 35.0 ml. of the CMP vehicle, giving a concentration of 20.12 mg./ml. of prednisolone in CMP. For analysis, 0.10-ml. samples of this solution were dissolved in 200 ml. of 95% ethanol, giving a calculated prednisolone concentration of 10.05 mcg./ml. of alcoholic solution.

A blank was prepared by dissolving 0.10 ml. CMP in 200 ml. of 95% alcohol. A reference standard was prepared by dissolving 60.0 mg. of USP reference standard prednisolone in 3.0 ml. CMP; 0.1 ml. of this mixture was added to 200 ml. 95% ethanol, giving a prednisolone concentration of 10.0 mcg./ml. of alcoholic solution. Blank, samples, and reference standard solutions all contained identical amounts of the CMP vehicle.

Twenty-milliliter aliquots of these solutions were assayed by the tetrazolium blue reaction (7) as follows: into each 20-ml. aliquot was pipeted 2.0 ml. of 0.5% tetrazolium blue in ethanol and 2.0 ml. of 10% tetramethylammonium hydroxide in ethanol. The solutions were mixed and the color allowed to develop for 90 min. in the dark.

The absorbances of reference standard and samples were read against the blank at a wavelength of 525 mμ in a Beckman DB-G spectrophotometer, with attached Sargent recorder, model SRL. After initial triplicate assays of the prednisolone–CMP preparation, the solution was incubated at 60° in a stoppered bottle. Samples (0.10 ml.) in triplicate were removed for assay at intervals of 24 hr., 5 days, 7 days, and 14 days. One additional sample was assayed after 6 weeks of incubation at 60°.

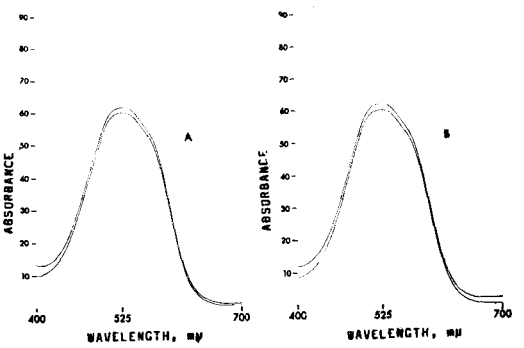

*Fig. 1—Stability of prednisolone after incubation at 60° in CMP (camphor—*m*-cresyl acetate—*p*-chlorophenol) compared to nonincubated USP reference standard prednisolone, as shown by tetrazolium blue assay. Key: upper curves, prednisolone; lower curves, nonincubated prednisolone; A, 24 hr.; B, 2 weeks.*

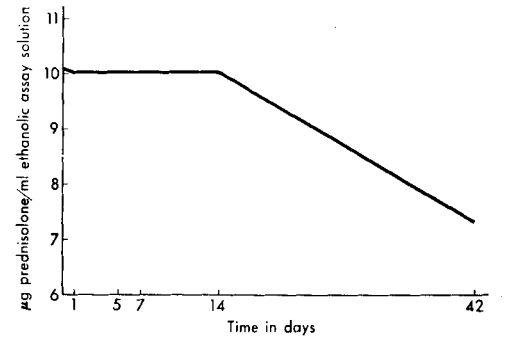

*Fig. 2—Stability of prednisolone in CMP (camphor—*m*-cresyl acetate—*p*-chlorophenol) incubated at 60°.*

RESULTS

Assay of the preincubation mixture gave a prednisolone concentration of 10.10 mcg./ml. of alcoholic solution. Assays after incubation at 60° for periods of 24 hr. to 2 weeks ranged from 10.03 to 10.05 mcg./ml. consistently (Fig. 1). After 6 weeks incubation of the prednisolone–CMP solution, the concentration of prednisolone was found to have dropped to 7.31 mcg./ml. of alcoholic assay solution (Fig. 2).

DISCUSSION

Prednisolone has been shown to be relatively unstable in aqueous suspensions at 35° (1), and even less stable when incubated at 35° in the presence of alkali (2) and trace metals (3). At pH 8, Oesterling and Guttman (3) obtained approximately a sevenfold increase in degradation rate when the temperature was increased from 35° to 70°. Thus the chief causes of prednisolone breakdown in pharmaceutical preparations have been shown to be alkalinity and trace metals, with degradation increasing as the temperature increases. In the organic vehicle, CMP, these catalysts of prednisolone degradation are absent and the preparation was found to be very stable at 60° for at least 2 weeks. Oesterling and Guttman (3) have shown prednisolone to be quite stable below pH 5. Thus good stability might be expected, since CMP is slightly acidic, due to the *p*-chlorophenol. It is suggested that the combina-

tion of 1% prednisolone in CMP, which is used in dentistry as a pulp-capping agent, would have a shelf-life of 2 to 5 years.

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Keyphrases

Prednisolone—stability in CMP
Degradation—prednisolone
Blue tetrazolium reaction—assay
IR spectrophotometry—analysis

Aster pilosus (*Compositae*) I. Isolation of Hyperoside (Quercetin-3-β-D-mono-galactoside) from the Leaves

By NORMAN R. FARNSWORTH, HILDEBERT WAGNER, LUDWIG HÖRHAMMER, and HANNS-PETER HÖRHAMMER

Aster pilosus **leaves were investigated and found to contain several flavonoids, as evidenced by thin-layer chromatography. Purification of a crude ethanol extract, followed by polyamide column chromatography, resulted in the isolation of one of the flavone constituents, which was found to be identical with hyperoside (quercetin-3-β-D-mono-galactoside).**

ALTHOUGH SEVERAL HUNDRED SPECIES of the genus *Aster* have been botanically described, relatively little is known of the phytochemical constituents of this group of plants. *Aster tataricus* roots have yielded the triterpenes friedelin, epifriedelanol, and shionone, in addition to astersaponin (hederagenin glucoside) (1–6) and the flavone quercetin (7). Quercetin, carotenoids, and galactose have also been detected in the pollen of this species (8). The latter three compounds have similarly been detected in the pollen grains of *Aster yomena* and *A. ageratoides* var. *ovatus* (8). *Aster tripolium* roots have yielded the polyacetylene compound 2-*trans*:8-*trans*-matricarianol (9) and *A. spinosus*, *cis*-lachnophyllum ester (10). Also, 2-*cis*: 8-*cis* matricaria ester has been isolated from the roots of *A. mongolicus*, *A. lautureanus*, and *A. novae angliae* (9), and angelic acid ester from *A. novae belgii* (11). Delphinidin diglycosides have been detected in the flowers of *A. amellus* var. *riverslea* and *A. sinensis* (12), cyanidin-3, 5-dimonoside in the leaves of *A. ericoides* (13), and callistephin (pelargonidin-3-glucoside), as well as chrysanthemin (cyanidin-3-glucoside), have been isolated from *A. chinensis* (14). A number of volatile oil constituents has also been identified in *Aster indicus* by means of gas chromatography (15).

Several *Aster* species have been used as folkloric remedies in the treatment of skin diseases (*A. novae angliae*) (16), rectal disorders, angina, eye inflammation, and stomach acidity (*A. amellus*, *A. tripolium*) (16), syphilis, bone caries, and coughs. (*A. bakerianus*) (17), intestinal parasites, and abdominal pain (*A. erigeroides*, *A. filifolius*) (17), and headache (*A. muricatus*) (17). In addition, various species have been recorded as being toxic to livestock, having strong emetic and purgative activity, producing depression, and having antiseptic properties (16, 17).

On the experimental side, *A. pilosus* var. *demotus* (18), *A. scaber* (19), *A. tataricus* (19), and *A. japonicus* (20) have been reported to elicit antitumor activity, whereas *A. divaricatus* extracts have shown strong inhibitory action against *Mycobacterium tuberculosis* (21). Defatted ethanol extracts of several native *Aster* species have been shown to elicit varying degrees of central system depression and/or autonomic activity in mice (22) and *A. divaricatus* is inhibitory for the pseudorabies virus in tissue culture tests (22).

No definitve phytochemical studies have been reported in the literature for *Aster pilosus* and biological activity reports on this species are limited (22–24).

On the basis of a lack of phytochemical information on *A. pilosus*, and because of interesting folkloric uses recorded for this genus of plants, a preliminary chemical study of the leaves was initiated.

EXPERIMENTAL

Plant Material—The plant material used in this investigation was the dried leaves of *Aster pilosus* Willd. (*Compositae*), collected during September 1966 at Pittsburgh, Pa. and authenticated by Dr. L. K. Henry, Carnegie Museum, Pittsburgh, Pa.

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