acid and 1 ml. of this solution was added to each of the standard alkaloid test reagents used.

RESULTS AND DISCUSSION

Since many substances can interfere with the alkaloid test reagents these should be taken only as an indication of the possible presence of alkaloids. Ten of the 28 plants examined had four or five positive alkaloid tests out of the five run and it would appear that these materials almost certainly contain alkaloids (Table I.). Seven additional plant extracts had three positive tests and thus these could be considered as having a high probability of containing alkaloids. As might be expected, the petroleum ether extracts contained no alkaloids and thus the petroleum ether extract followed by an ethanol extract provides a good preliminary separation of some of the nonalkaloid components from the alkaloids.

Based on the standards of activity set by the Walter Reed Army Medical Center, none of the extracts tested could be considered as being active against *P. berghei* in mice (Table II.). Only 4 of the 13 extracts screened for antimalarial activity extended the survival time more than 1 day beyond the control and the most active, *Cinnamomum mercadoi vidal*, had only about one half the extension in survival time needed to be considered an active compound.

Five (serial No. 2, 4, 5, 23, and 25) of the original ethanol extracts had an activity worthy of note against the KB cell culture (Table III.). In the case of *Sphaeranthus africanus*, this activity was also present in the petroleum ether extract but not in the ethanol extract which followed the petroleum ether extract. With *Anona squamosa* the activity was not present in the petroleum ether extract, while both *Calophyllum blancoi* and *Straphanthus cumingii* had activity in the ethanol extract which followed the petroleum ether extraction. None of the extracts had outstanding activity against any of the animal tumors but five (serial No. 2, 9, 16b, 23, and 28a) had at least moderate activity against two animal systems each. Two (serial No. 21 and 28b) had moderate activity against one system each. It is of interest to note that only two of the compounds active in the cell culture screen had any appreciable activity in animal screens, *A. squamosa* and *Phaseolus aureus*.

SUMMARY

Of 28 Philippine plants studied, between 10 and 17 could be considered to contain alkaloids.

None of the extracts screened had any appreciable antimalarial activity, although one had a slight indication of activity.

Five of the plants were active against KB cell culture, and a number had moderate activity against a variety of animal cancers.

Improvement of the Color Stability of Parenteral Solutions of Papaverine Hydrochloride

By D. E. GRIFFITH

Disodium ethylenediaminetetraacetate (EDTA) 0.005 per cent successfully inhibits color formation in parenteral papaverine hydrochloride solutions.

PAPAVERINE HYDROCHLORIDE, one of the alkaloids of opium, is used primarily as an antispasmodic for smooth muscle, including arterial (1). It has also been prepared synthetically, and is chemically the hydrochloride of 6, 7, 3′, 4′-tetra-methoxy-1-benzylisoquinoline (1).

The chemical stability at room temperature of papaverine hydrochloride injection, as determined by the N.F. XII alkaloid extraction method, is extremely good. There is no detectable loss in potency in 4 years. However, an amber color starts to form within 1 year at 25° and within 1 month at 37°.

Disodium ethylenediaminetetraacetate (EDTA or sodium edetate) is a sequestering agent which has been used in medicine primarily as a binding agent in heavy metal poisonings; the usual dose being 75 mg./Kg. of body weight of a 20% solution (2). Salk poliomyelitis vaccine has been stabilized with sodium edetate, 7 : 20,000.[1]

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[1] Eli Lilly and Co.

The use of sodium edetate to prevent discoloration of pharmaceuticals is recorded several times in the literature (3–8). Discoloration has been prevented in morphine, phenylephrine, sodium sulfacetamide, procaine, and ajmaline solutions. Amounts of sodium edetate used vary from 0.005–0.04% in the references cited.

The purpose of this study was to inhibit the color formation of papaverine hydrochloride injection without changing the chemical stability. The approach was through use of sodium edetate while controlling all pertinent variables.

EXPERIMENTAL

The experiments were designed to determine the effect on papaverine hydrochloride color stability of (*a*) various concentrations of sodium edetate, (*b*) the container, (*c*) light and atmosphere, and (*d*) different papaverine hydrochloride raw material lots.

The experimental solutions were formulated with conventional laboratory apparatus, reagent grade chemicals, and water for injection U.S.P. The solutions were sterilized by either autoclaving or sterile filtration by Selas 02 candles. The glass-sealed ampuls and rubber-stoppered vials were flint type I glass. They were washed on conventional ampul washing machines and sterilized by

TABLE I—RELATIVE COLOR STABILITY OF PAPAVERINE HYDROCHLORIDE PARENTERAL SOLUTIONS WITH AND WITHOUT SODIUM EDETATE—TIME TO DISCOLOR TO A.P.H.A. COLOR STANDARD 15[a] PAPAVERINE HYDROCHLORIDE WITH NITROGEN ATMOSPHERE

Temp., °C.	No EDTA	0.005% EDTA
50	2 wk.	5 mo.
37	1 mo.	9 mo.
25	12 mo.	Colorless to date (2 yr.)

[a] Standards of American Public Health Association using solutions of platinum chloride in water. A.P.H.A. 15 is a pale yellow color.

dry heat. The glass-sealed ampuls were sealed by pull-type seals. The rubber-stoppered vials were sealed with West natural rubber stoppers and aluminum seals. Aging tests were conducted at room temperature and in ovens at 37° and 50°. Light exposure consisted of exposure to direct sunlight in a window facing south, and also by exposure on a shelf to fluorescent lights approximately 10 ft. away. Color was noted visually by observing against a white background. The N.F. XII assay procedure for papaverine hydrochloride was used in all cases. Samples of papaverine hydrochloride containing 0%, 0.005%, 0.01%, and 0.02% sodium edetate were formulated.

Samples of papaverine hydrochloride with 0.005% and no sodium edetate were stored in glass-seal ampuls and rubber-stoppered vials. Two lots of papaverine hydrochloride raw material were used for the experiments. Some ampuls of each section were exposed to light. Some contained a nitrogen atmosphere. All sections were exposed to 25, 37, and 50° temperatures. The samples were observed weekly to 2 months, then monthly to the point of discoloration.

DATA AND RESULTS

Oxygen in the air and light detract from color stability of papaverine hydrochloride solutions. The effect, however, is considerably less for sodium edetate formulations. Temperature has less effect on discoloration with the sodium edetate formulations, but still is a significant factor. It was found that color inhibition was maximum at 0.005% sodium edetate and was not improved by increasing the concentration of sodium edetate. The discoloration noted in glass-seal ampuls and rubber-stoppered vials was the same, indicating no effect from the package. No difference was seen between either of the lots of papaverine hydrochloride raw material. The type of sterilization (filtration *versus* autoclave) did not affect color formation. The amount of improvement of color stability can be seen in Table I.

CONCLUSIONS

Disodium ethylenediaminetetraacetate (sodium edetate or EDTA) successfully inhibits color formation in injectable solutions of papaverine hydrochloride at a concentration of 0.005%. Protection from light and oxygen offer additional protection. Chemical potency is still at 100% at 37° at 18 months. It is believed that the mechanism of protection is through sequestering trace quantities of heavy metals present, such as iron, which may react with chloride ions in the solution at the low pH (3.0–4.0) of these solutions. That this is so was determined by adding a tiny piece of iron oxide to each of several vials of papaverine hydrochloride which turned dark amber soon after.

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New Compounds: *N*-Substituted Tetrahydroisoquinolines

By JOSEPH SAM*

The Mannich, sodium borohydride reduction, and esterification reactions were utilized for the preparation of several *N*-substituted tetrahydroisoquinolines. Aminolysis of ethyl cyanoacetate with tetrahydroisoquinoline provided the corresponding cyanoacetamide.

SEVERAL *N*-SUBSTITUTED tetrahydroisoquinolines were prepared and screened for biological activity. The Mannich reaction with *p*-chloro- and *p*-methoxyacetophenone yielded two tetrahydroisoquinolino-ketones (I). Mannich and Lammering (1) earlier had described the preparation of unsubstituted I. The sodium borohydride reduction of I resulted in II; treatment of II*a* with 3,4,5-trimethoxybenzoyl chloride provided the corresponding ester.

The aminolysis of ethyl cyanoacetate with tetra-

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