Investigations of Philippine Plants for Alkaloids, Antimalarial Agents, and Antineoplastic Agents

By F. D. POPP, J. M. WEFER, G. ROSEN, and A. C. NOBLE

Extracts of 28 different Philippine plants were prepared and the extracts tested for alkaloids. The extracts were also screened, in many cases, for antimalarial and antineoplastic activity. In some cases preliminary fractionation into petroleum ether soluble and ethanol soluble extracts were made.

THE IMPORTANCE of screening plant materials for various chemical constituents of medicinal importance is well established. Plants have provided many drugs which are being used for the prevention or cure of diseases or which have been the basis for synthetic drugs. This work was directed to the search for new drugs which may find application as antineoplastic agents or as antimalarial agents.

TABLE I—ALKALOID TEST RESULTS

No.	Name	Plant Part[a]	Extract[b]	Alkaloid Tests[c] A	B	C	D	E
1	*Ageratum conyzoides* (*Compositae*)	S, L	Alc	−	+	+	+	+
1a			PE	−	−	−	−	−
1b			Al/PE	+	+	+	+	+
2	*Anona squamosa* (*Annonaceae*)	L	Alc	+	+	+	−	−
2a			PE	+	−	−	−	−
2b			Al/PE	+	+	−	−	−
3	*Asclepias curassavica* (*Asclepiadaceae*)	W	Alc	−	+	−	+	+
4	*Blumea balsamifera* (*Compositae*)	L	Alc	+	+	−	+	+
5	*Calophyllum blancoi* (*Guttiferae*)	B	Alc	−	+	−	−	−
5a			PE	−	−	−	−	−
5b			Al/PE	−	+	−	−	−
6	*Calophyllum inophyllum* (*Guttiferae*)	B	Alc	−	+	−	−	−
7	*Cassia siamea* (*Leguminosae*)	L, P	Alc	−	+	−	+	+
8	*Cinnamomum mercadoi vidal* (*Lauraceae*)	L	Alc	−	+	+	−	−
8a			PE	−	−	−	−	−
9	*Cucumis melo* (*Tournaceae*)	L	Alc	−	−	−	−	−
10	*Dioscorea hispida* (*Dioscoreaceae*)	T	Alc	+	+	+	+	+
11	*Dolichos lablab* (*Leguminosae*)	L	Alc	+	+	+	+	+
12	*Eclipta alba* (*Compositae*)	S, L	Alc	−	+	−	+	+
13	*Erythrina variegata* (*Leguminosae*)	L	Alc	−	+	−	+	+
14	*Eugenia jambolana* (*Myrtaceae*)	S	Alc	−	−	−	−	−
15	*Gammatophyllum scriptum* (*Orchidaceae*)	Bl	Alc	−	−	−	−	+
16	*Geodorum nutans* (*Orchidaceae*)	Bl	Alc	−	+	−	+	+
16a			PE	−	−	−	−	−
16b			Al/PE	−	+	−	+	+
17	*Hernandia ovigera* (*Hernandiaceae*)	L	Alc	+	−	−	−	−
18	*Hibiscus rosa-sinensis* (*Malvaceae*)	L	Alc	+	+	+	+	+
19	*Ipomoea pes-caprae* (*Convolvulaceae*)	L	Alc	−	+	−	+	+
20	*Kibatalia gitingensis* (*Apocynaceae*)	B	Alc	+	+	+	+	+
21	*Mitragyna rotundifolia* (*Rubiaceae*)	L	Alc	+	+	+	+	+
22	*Nerium oleander* (*Apocynaceae*)	L	Alc	+	+	−	−	−
23	*Phaseolus aureus* (*Leguminosae*)	L	Alc	−	−	−	−	+
24	*Pseudelephantopus spicatus* (*Compositae*)	S, L	Alc	−	−	−	−	−
25	*Sphaeranthus africanus* (*Compositae*)	W	Alc	−	+	−	−	+
25a			PE	−	−	−	−	−
25b			Al/PE	−	+	−	−	+
26	*Strophanthus cumingii* (*Apocynaceae*)	S	Alc	−	+	+	+	+
26a			PE	−	−	−	−	−
26b			Al/PE	−	+	−	+	+
27	*Tabernaemontana pandacaqui* (*Apocynaceae*)	B	Alc	+	+	+	+	+
28	*Vitex trifolia* (*Verbenaceae*)	L	Alc	+	+	+	+	+
28a			PE	−	−	−	−	−
28b			Al/PE	+	+	+	+	+

[a] S, stems; L, leaves; W, whole plant; B, bark; P, pods; T, tubers; Bl, bulbs. [b] Alc, 80–90% ethanol extract; PE, petroleum ether extract; Al/PE, extract of petroleum ether extracted material with ethanol. [c] A, Hager's reagent (picric acid); B, Sonnenschein's reagent (phosphomolybdic acid); C, Mayer's reagent (mercuric potassium iodide); D, Dragendorff's reagent; E, Scheibler's reagent (phosphotungstic acid).

EXPERIMENTAL

Materials—All plant materials were collected, identified, and supplied in the air-dried state by Dr. Demetrio Mendoza, Philippine National Museum, Manila.

Received April 21, 1967, from the Department of Chemistry, Clarkson College of Technology, Potsdam, NY 13676
Accepted for publication June 12, 1967.
The authors thank Dr. D. Mendoza, the National Museum, Manila, for assistance in obtaining the plant materials and for many helpful suggestions and Dr. L. Rane and Dr. J. L. Hartwell for assistance in having the extracts screened.
This investigation was supported in part by grant T-234 from the American Cancer Society and grant CA-07891 from the National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

TABLE II—MALARIA SCREENING DATA[a]

No.[b]	Dose,[c] mg./Kg.	Survival Time, Days[d] Test	Control	Notes
2a	160	6.4	6.1	Toxic at 640 mg./Kg.
3	640	8.0	6.2	Some toxicity
4	640	7.2	6.4	
6	640	6.8	6.3	
7	1280	8.0	6.8	
8	40	9.6	6.4	10.0 survival with some toxicity at 160 mg./Kg.
8a	160	6.4	6.2	
10	640	8.0	6.4	
13	640	7.0	6.3	Some toxicity
15	640	7.6	7.0	
17	640	6.2	6.1	
21	640	6.2	6.1	
22	640	6.4	6.1	
23	160	7.4	7.2	
27	40	6.8	6.1	

[a] Samples sent to Dr. L. Rane, University of Miami, for screening; results received from Walter Reed Army Medical Center. Screening against *Plasmodium berghei* in mice infected with a lethal dose 3 days prior to administration of the extract. [b] Refer to Table I. [c] At least three different doses were given for each extract, the highest nontoxic dose is recorded here. [d] A compound is considered to be active when the survival time of the test is more than twice the survival time of the control.

Preparation of Extracts—The air-dried plant materials were ground and extracted in a Soxhlet extractor until a fresh extract had relatively little color to it. All plant materials were extracted with 80–90% ethanol and the ethanol extract was concentrated to dryness *in vacuo*. The residue from this concentration was tested for alkaloids and submitted for screening.

In selected cases a fresh batch of air-dried plant materials were extracted first with petroleum ether (naphtha) and then with 80–90% ethanol. These individual extracts were concentrated as noted above.

Screening—Most of the concentrated extracts were submitted to Dr. J. L. Hartwell, Cancer Chemotherapy National Service Center (CCNSC), for anticancer screening under the CCNSC standard protocol, and the anticancer results included in this paper were supplied by the CCNSC. Many extracts were also submitted to Dr. L. Rane, University of Miami, for screening against *Plasmodium berghei* in mice. The antimalarial results included in this paper were supplied by the Walter Reed Army Medical Center.

Alkaloid Tests—The concentrated extract (0.1 Gm.) was dissolved in 10 ml. of 0.5% hydrochloric

TABLE III—CANCER SCREENING DATA[a]

No.[b]	KB Cell Culture[c] ED_{50}, mcg./ml.	Slope	T/C (%)/Dose (mg./Kg.) LE[d]	SA[e]	DL[f]	WM[g]	LL[h]
1a			106/400	...	...	106/25	...
1b	5.4×10^{1}	−1.0	...	...	...	...	...
2	9.4×10^{-2}	−0.2	123/5	75/6	...	100/5	...
2a	2.5×10^{1}	−0.4	110/18	...	...	106/25	...
4	1.7×10^{1}	−0.4	...	...	...	...	...
5	9.6×10^{0}	−0.6	...	...	...	...	...
5b	3.0×10^{0}	−0.7	...	...	...	...	...
6	M1.0 × 10^{2}		...	...	...	...	...
7	M1.0 × 10^{2}		...	...	100/480	...	...
8	3.3×10^{1}	−0.6	...	...	...	...	...
9	M1.0 × 10^{2}		100/400	62/500	...	56/400	...
10	M1.0 × 10^{2}		...	...	...	...	...
11	1.2×10^{2}	−0.5	...	...	...	...	...
12	3.6×10^{1}	−1.0	...	...	...	...	...
13	6.0×10^{1}	−0.6	...	...	...	...	...
14	9.1×10^{1}	−0.7	...	...	100/320	...	...
15	M1.0 × 10^{2}		95/400	98/500	...	100/400	...
16	M1.0 × 10^{2}		93/175	154/500	...	...	93/350
16a	M1.0 × 10^{2}		103/100	122/125	...	117/100	...
16b	7.9×10^{1}	−0.7	94/400	75/500	...	...	61/400
18	M1.0 × 10^{2}		...	...	...	...	...
19	4.2×10^{1}	−0.6	...	...	...	...	...
20			...	...	104/640	...	...
21	M1.0 × 10^{2}	−0.4	92/400	...	...	76/400	...
23	2.7×10^{1}	−1.2	104/100	44/125	...	...	51/100
24			93/500	...	...	102/500	...
25	1.3×10^{1}	−0.4	...	...	107/640	...	...
25a	2.8×10^{0}	−0.3	...	...	...	...	...
25b	M1.0 × 10^{2}		...	...	...	...	...
26			...	...	104/20	...	...
26a	M1.0 × 10^{1}		...	...	...	...	...
26b	2.7×10^{1}	−1.1	...	...	...	...	...
27	4.5×10^{1}	−0.8	100/100	...	...	85/400	...
28			...	...	104/320	...	...
28a	M1.0 × 10^{1}		90/80	62/100	...	...	61/80
28b	1.7×10^{1}	−0.4	92/200	47/500	...	...	135/100

[a] Screening results received from Cancer Chemotherapy National Service Center (CCNSC), National Institutes of Health. All screening done by CCNSC contract screeners according to standard CCNSC screening protocol. [b] Refer to Table I. [c] ED_{50}, dose that inhibits growth to 50% of control growth. Slope, difference in result for a tenfold difference in dose. [d] L-1210 lymphoid leukemia. [e] Sarcoma 180. [f] Dunning leukemia (solid). [g] Walker carcinosarcoma 256 (intramuscular). [h] Lewis lung carcinoma.

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]

Received March 22, 1967, from the Research and Development Laboratories, Eli Lilly & Co., Indianapolis, IN 46206

Accepted for publication June 19, 1967.

[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