Canscora decussata (Gentianaceae) Xanthones III: Pharmacological Studies

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Abstract □ Pharmacological studies of mangiferin, the major and most polar xanthone of *Canscora decussata* (Family Gentianaceae), and some of the less polar xanthonic constituents of the roots are reported. Mangiferin showed definite signs of CNS stimulation. Hydrocholeretic effect, cardiostimulant effect, and potentiation of subanalgesic doses of morphine are also demonstrated. The less polar xanthones, from the petroleum extract of the roots, elicited only a weak CNS depressant activity. Selected pharmacological screening of the water-soluble xanthones indicated that this fraction contained the active principle(s) of the plant.

Keyphrases □ *Canscora decussata* (Gentianaceae)—pharmacological study of xanthone constituents □ Xanthones from *Canscora decussata*—pharmacological studies □ Mangiferin—xanthones from *Canscora decussata*, pharmacological activity □ Medicinal plants—*Canscora decussata* xanthones, pharmacological activity

Synthetic xanthones carrying basic substituents are known to possess oral schistosomicidal activity in experimental animals (1–5). Several investigations have also shown: (*a*) an analeptic effect in the treatment of respiratory depression, (*b*) a CNS stimulant effect (4), and (*c*) a tuberculostatic effect with synthetic xanthones (3, 4, 6). 1,3,8-Trihydroxyxanthone, a degradation product of sterigmatocystin, was reported (6) active at a dilution of 1 in 80,000 against *Mycobacterium tuberculosis*. Sterigmatocystin, occurring in *Aspergillus versicolor*, had virtually no tuberculostatic effect. Limited pharmacological studies with mangiferin (C_2-β-glucoside of 1,3,6,7-tetrahydroxyxanthone), which occurs widely among angiosperms (7), was reported (8) to produce increased diuresis in the adrenalectomized rat and a cardiotonic effect in isolated frog heart. Except in these two instances, data on the pharmacological activity of naturally occurring xanthones are lacking in the literature.

It was, therefore, considered worthwhile to determine the pharmacological profile of the polyoxygenated xanthones of *Canscora decussata* (Family Gentianaceae) (9–11). The plant is used for a variety of purposes in the Ayurvedic system of medicine (12). The root extracts are used as a laxative, as a diuretic, for liver troubles, and for tuberculosis treatment but its most significant use is in mental disorders. The aerial portions are used in the treatment of insanity, epilepsy, and nervous debility. Since polyoxygenated xanthones are the major chemical constituents of the plant (over 90% of the chemical characters isolated), search for its active principle(s) in these entities seemed warranted. The present paper reports pharmacological studies with mangiferin, the major and most polar xanthone of the plant, and some of the less polar xanthones from the petroleum extracts of the roots. Selected pharmacological screening of the water-soluble xanthones of *C. decussata* is also reported.

EXPERIMENTAL

Animals—Albino mice (20–30 g.) and albino rats (150–200 g.) of either sex, bred from C.D.R.I. strains, were used. They were fed[1] and housed for about a week in the laboratory animal house prior to any experiment. Healthy mongrel dogs (10–15 kg.) were procured locally and kept in the laboratory animal house for at least 3 days prior to experimentation.

Drug Preparation and Administration—The solubility of the xanthones was incomplete even at a concentration of 1 mg./ml. in distilled water. Unless otherwise stated, the test compounds were injected intraperitoneally in a vehicle of 2% Na_2CO_3 solution (resultant pH about 8), 1 ml./kg., for rats and mice. The same volume of 2% Na_2CO_3 solution was injected intraperitoneally to the control animals.

Gross Behavior—The test drugs, mangiferin or the total xanthones from the petroleum ether extract of *C. decussata*, were administered to groups of five mice each in doses of 25, 50, or 100 mg./kg. i.p. Gross behavioral changes were recorded at 15, 30, 60, and 120 min. after drug administration and were compared with the vehicle control group. In another set of experiments, the animals were pretreated with chlorpromazine (5 mg./kg. i.p.) 30 min. before administration of 50 mg./kg. of mangiferin.

Pentobarbital Sleeping Time—Mangiferin or the total xanthones, 50 mg./kg., or an equivalent volume of vehicle, was injected 30 min. before the administration of 30 mg./kg. i.p. of pentobarbital to groups (10 animals each) of albino rats. The duration of sleep was assessed as the time between the loss and return of the righting reflex. Results are expressed as percent increase in sleeping time of drug-pretreated rats *versus* those of the control group.

Effect on Subnarcotic Dose of Ethanol—Thirty minutes after injection of 50 mg./kg. i.p. of mangiferin or the total xanthones, or an equivalent volume of vehicle, ethanol as a 20% solution was injected at a dose of 4 g./kg. i.p. to groups of 10 mice each. This dose of ethanol caused a loss of the righting reflex in a maximum of 5% of the nontreated animals (13). The number of mice that lost their righting reflex after pretreatment with the test compounds was determined using an "all or none" criterion.

Reserpine-Induced Ptosis and Depression—Mangiferin or the total xanthones in doses of 25, 50, or 100 mg./kg. i.p., or an equivalent volume of vehicle, was administered to groups of 10 mice each 30 min. prior to injection of reserpine, 5 mg./kg. i.p. The degree of ptosis in the different groups was assessed 150 min. after reserpine administration, using a rate scaling in which 4 represents a normal palpebral opening and scores of 3, 2, 1, and 0 represent various degrees of response from slight to complete closure of eyelids. The PD_{50} was determined graphically by the method of Litchfield and Wilcoxon (14) as the dose at which 50% of the animals had eyelid opening scores of 3 or more. Antagonism to reserpine-induced sedation and locomotor activity were observed qualitatively, and no attempt was made to quantitate them.

Amphetamine Group Toxicity—Mangiferin or the total xanthones in doses of 25, 50, and 100 mg./kg. were administered to groups of 10 albino rats each; 30 min. later, 40 mg./kg. i.p. of amphetamine was administered to all the animals. The animals were then segregated in identical wire mesh cages (25 × 25 × 40 cm.). Mortality in each group was counted 5 hr. later. The ED_{50} was determined graphically, by the method of Litchfield and Wilcoxon (14), as the dose that caused 50% potentiation of mortality as compared to the control group. The experiments were conducted at 22 ± 1.0°.

Perfused Frog Heart—The effect of graded doses of mangiferin or the total xanthones was studied on perfused frog heart. Pro-

[1] Anidiet A and B, Chalsea Chemicals.

pranolol (20 mcg.) was used as the blocking drug. In the same experiments, the effect of mangiferin or the total xanthones was noted on a heart that was rendered hypodynamic by perfusing with Ringer's solution containing one-fourth of the usual calcium chloride. The effect of vehicle was also assessed. Ten experiments were conducted.

Analgesic Effect—The analgesic effect of mangiferin or the total xanthones in doses of 10, 20, or 40 mg./kg. i.p. was assessed by the rat tail flick method of Davies *et al.* (15) in groups of 10 albino rats each. In a separate experiment, 40 mg./kg. i.p. of mangiferin or the total xanthones, or an equivalent volume of vehicle, was administered to groups of 10 albino rats each. Thirty minutes later, all of the rats were injected with a subanalgesic (2 mg./kg. i.p.) dose of morphine hydrochloride (16). The percent potentiation of analgesia induced by the test drugs as compared to the control group was determined.

Anticonvulsant Effect—Mangiferin or the total xanthones in doses of 25, 50, or 100 mg./kg. i.p., or an equivalent volume of vehicle, was injected into groups of 10 albino rats each. Thirty minutes later, all of the animals were tested for anticonvulsant activity by the maximal electroshock techique (17) or by the maximal pentylenetetrazol-induced convulsion technique (18).

Diuretic Effect—Diuretic activity of the test compounds was assessed by a modified method of Lipschitz *et al.* (19). Male albino rats (150 ± 10 g. on test day) were hydrated orally with normal saline (25 ml./kg.) and starved for 18 hr. Water was allowed *ad libitum* but was removed 1.5 hr. before test time. At test time, individually weighed rats were again hydrated (25 ml./kg.) with normal saline and test compounds were incorporated in this water load. Groups of five animals for each dose level of the test drugs (mangiferin or the total xanthones), with one group serving as the untreated control (saline and vehicle only), were kept in metabolic cages. Diuretic response for a 6-hr. test period was calculated in terms of percent increase in urine excretions in excess of the control. The groups were crossed over after a week's interval.

Effect on Blood Pressure, Respiration, and Intestine *In Situ* of Anesthetized Dog—Mongrel dogs, of either sex, were anesthetized with pentobarbital (35 mg./kg. i.p.). Carotid blood pressure, respiratory excursions, and intestinal movements were recorded by routine methods. Drugs were injected through the cannulated femoral vein. Graded doses of mangiferin or the total xanthones, or an equivalent volume of vehicle, was injected intravenously; their effects on blood pressure, respiration, and intestinal movements were recorded on a smoked kymograph. Five experiments were conducted.

Effect on Biliary Flow—The effect of the test compounds on biliary flow was assessed by the method of Bhattacharya *et al.* (20). Mongrel dogs of either sex were anesthetized with pentobarbital (35 mg./kg. i.p.). The abdomen was opened by a midline incision, the cystic duct was ligated, and the bile duct was cannulated close to its termination. The bile secretion was measured at 30-min. intervals; when at least two 30-min. bile samples were approximately equal, the test compounds (20 mg./kg.) or an equivalent volume of vehicle were injected through the cannulated femoral vein. The bile flow was again measured at 30-min. intervals for 240 min. The percentage increase in bile volume over the preinjection control level was determined. Five dogs were used for the test compound.

Acute Toxicity—Acute toxicity of the test compounds was investigated in albino rats after intraperitoneal administration. Four animals were tested at each dose, and four doses (100, 200, 500, and 1000 mg./kg.) of each drug were tested. The LD_{50} was determined by the method of Litchfield and Wilcoxon (14). Surviving animals were kept under observation for a further period of at least 5 days.

RESULTS AND DISCUSSION

The vehicle (2% sodium carbonate solution) did not show any pharmacological activity in the volume used.

Definite signs of CNS stimulation were observed with mangiferin in the gross behavioral studies. In doses of 50 and 100 mg./kg., mangiferin induced tremors, piloerection, compulsive gnawing, and increased motor activity in all of the test animals. The behavioral changes reached a peak by 30 min. of drug administration, were sustained up to 60 min., and then gradually declined by 120 min. All these effects were blocked by chlorpromazine pretreatment. The total xanthones from the petroleum extract did not elicit any hyperactivity, but the animals showed overt signs of CNS depression (decreased motor activity, sedation, and diminished response to external stimuli). Both mangiferin and the total xanthones (50 mg./kg.) significantly potentiated ($p < 0.05$) pentobarbital sleeping time. Rats pretreated with mangiferin and the total xanthones slept for 56.2 ± 7.8 and 58.7 ± 6.7 min. (±*SEM*), respectively, as compared to 36.2 ± 5.3 min. (±*SEM*) sleeping time in the control group. However, only mangiferin (50 mg./kg.) significantly ($p < 0.001$) potentiated the effect of a subnarcotic dose of ethanol. In this dose, 60% of the treated mice showed a loss of the righting reflex as against none in the untreated control group. The total xanthones had no demonstrable effect in this dose.

Mangiferin exhibited a dose-related inhibition of reserpine-induced ptosis, sedation, and depression of locomotor activity in the doses studied. The PD_{50} against reserpine-induced ptosis was 42.4 mg./kg. (31.5–49.8 mg./kg. at 95% fiducial limits). The total xanthones did not show any significant activity against these parameters. Mangiferin also produced a dose-related potentiation of amphetamine group toxicity. The ED_{50} was determined as 76.2 mg./kg. (56.5–94.0 mg./kg. at 95% fiducial limits). The total xanthones did not exhibit any significant effect in this parameter.

The behavioral effects of mangiferin together with its ability to potentiate pentobarbital-, ethanol-, and amphetamine-induced pharmacological effects indicate the potential antidepressant nature (13) of the compound.

Both mangiferin and the total xanthones produced a transient positive inotropic effect on perfused frog heart in doses of 1–2 mg. Since the effect was not blocked by propranolol, it was a direct cardiostimulant action. A similar transient positive inotropic effect was observed in hypodynamic frog heart.

Mangiferin or the total xanthones did not elicit any analgesic activity of its own in doses up to 40 mg./kg. However, in this dose, mangiferin significantly ($p < 0.001$) potentiated the analgesia produced by subanalgesic doses of morphine. In the vehicle-pretreated control group, the latent period of tail flick induced by a subanalgesic dose of morphine was 11.14 ± 0.25 sec. (±*SEM*), whereas in the mangiferin-pretreated (40 mg./kg.) group, the same dose of morphine (2 mg./kg.) induced a latent period of 17.30 ± 0.31 sec. (±*SEM*). The total xanthones did not produce any significant effect in this parameter.

Mangiferin or the total xanthones did not elicit any anticonvulsant activity against maximal electroshock and pentylenetetrazol-induced convulsion in doses up to 100 mg./kg. Likewise, these compounds had no significant diuretic effect up to dose levels of 100 mg./kg. No significant effects on the dog's carotid blood pressure, respiration, and intestinal movements were observed with mangiferin or the total xanthones up to a dose of 20 mg./kg.

Mangiferin produced a moderate increase in bile flow in doses of 20 mg./kg. From preinjection control levels, the bile flow started increasing after 30 min. of the drug administration (180% increase); by 60 min., it reached a peak effect (290% increase). This choleretic effect started waning by 90 min. (220%) and had almost passed off (56%) by 240 min. The increase of bile secretion at 30, 60, and 90 min. of drug administration, over the preinjection basal level, was statistically significant ($p < 0.001$). The total xanthones from the petroleum extract had no effect on bile flow up to a dose of 50 mg./kg.

The LD_{50} of mangiferin in albino rats (based on a total of 16 animals) was 365 mg./kg. (303–416 mg./kg. at 95% fiducial limits). The total xanthones in a dose range of 500–1000 mg./kg. caused no deaths in albino rats for a period extending 5 days after a single intraperitoneal injection.

Of the two test compounds, the pharmacological actions of mangiferin appear to justify the therapeutic uses of the plant *C. decussata* in some mental disorders (*e.g.*, melancholia). However, neither of the two test compounds seems to be the true active principle of the plant. This contention finds support from the fact that an aqueous solution (1 ml./25 g. of powdered plant material) of the residue, obtained from the alcoholic extract of the plant, on preliminary investigation was found to produce marked sedation and some degree of ptosis in albino rats. The total extract also elicited marked potentiation (110%, $p < 0.001$) of pentobarbital-induced hypnosis in doses of 0.3–0.9 ml./100 g. i.p. Consequently, the major active principle of this vegetable drug seems to be present in the total alcoholic extract. Further studies are currently underway to explore the nature of these active principles of *C. decussata*.

The present investigation failed to substantiate the diuretic effect reported with mangiferin (8). Also, the cardiostimulant effect of mangiferin on the frog's perfused heart was only a transient one.

The choleretic effect of mangiferin is consistent with the use of the plant extracts in liver diseases in the indigenous system of medicine. Pharmacological investigation with other chemical constituents (*e.g.*, alkaloids and tri- and monoterpenoids) (10) of *C. decussata* may reveal the justifiability of its uses in other clinical conditions.

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Anomalous Isolation of an Active Antitumor Alkaloid from a Fraction of *Catharanthus lanceus* Devoid of Anticancer Activity

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Abstract □ The highly active antitumor alkaloid leurosine was isolated from the leaf (C) fraction of *Catharanthus lanceus*. Previously, this (C) fraction had been shown to be devoid of antitumor activity against the P-1534 leukemia in DBA/2 mice. At present, no good explanation for this phenomenon can be made.

Keyphrases □ *Catharanthus lanceus*—isolation of leurosine from a previously inactive fraction, an antitumor alkaloid □ Leurosine—anomalous isolation from a previously inactive fraction of *Catharanthus lanceus* □ Antitumor alkaloids—isolation of leurosine from *Catharanthus lanceus*

The isolation of biologically active substances from plant materials, when guided by bioassay, is dependent on utilizing a reproducible biological test system that will detect the activity being sought. The most likely explanation for the presence of a biologically active substance in a crude extract devoid of the same activity is that the active substance is present at a concentration below the limit of detection. This paper reports how the leaf (C) alkaloid fraction from *Catharanthus lanceus* was subjected to a column chromatographic separation in which the highly active antitumor alkaloid leurosine was isolated in a yield of 0.28% of the (C) fraction. The crude alkaloid fraction previously had been shown to be inactive against the P-1534 leukemia in DBA/2 mice, a tumor system that is highly susceptible to the action of leurosine in the pure state.

EXPERIMENTAL

Isolation of Alkaloids from (C) Fraction—The method of preparation of the alkaloid (C) fraction from the leaves of *C. lanceus* Boj. ex A.DC (Apocynaceae) was previously reported (1). An aliquot of this fraction (92 g.) was dissolved in chloroform (1 l.) and filtered to remove a small amount of nonalkaloid material. To the filtrate was added an equal volume of 2% (w/v) aqueous tartaric acid solution, and the mixture was heated on a steam bath *in vacuo* until the chloroform was completely removed. The solution was filtered to remove 23.3 g. of insoluble residue, which was discarded. After cooling the filtrate, it was extracted three times with equal volumes of chloroform. The chloroform extracts were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness *in vacuo* to yield 61 g. of Fraction I alkaloids. The resulting aqueous phase was rendered alkaline to litmus paper with ammonium hydroxide and extracted several times with chloroform until all of the alkaloids were removed from the aqueous phase (negative Mayer's test). After combining the chloroform extracts, drying