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

over anhydrous sodium sulfate, filtering, and removing the chloroform *in vacuo*, a residue of 7.3 g. was obtained (Fraction II).

Fraction I (61 g.) was dissolved in benzene and added to the top of a chromatographic column filled with a benzene slurry of neutral alumina[1]. Elution was continued with benzene, and 500-ml. fractions were collected. Fractions 6–12 from the column were combined and taken to dryness (7.4 g.), and the residue was subjected to a gradient pH separation as previously described (2). The initial benzene extraction at pH 2.7 yielded 3.33 g. of alkaloid residue, which was chromatographed over a column of neutral alumina as previously described. Benzene was used as the eluent, and 200-ml. fractions were collected. Fractions 28–29 were combined, taken to dryness, and treated with hot methanol. On standing, crystals formed, which were dried *in vacuo* for 24 hr. Subsequently, these crystals were found to be identical in all respects with yohimbine, which was previously reported from this plant (1). Fractions 12–27 from the column were combined, taken to dryness *in vacuo*, and yielded 1.95 g. of alkaloid residue. This residue was rechromatographed over a column of neutral alumina as previously described. Benzene elution yielded a homogeneous material. Crystallization from ethanol yielded crystals that were identical in all respects with leurosine, which was previously isolated from the leaf alkaloid (A) fraction of this plant (1). A total of 0.255 g. of leurosine (base) was obtained.

Antitumor Testing—Extracts and alkaloids were assayed for activity against the P-1534 leukemia in DBA/2 mice according to protocols of the National Cancer Institute (3). The leaf crude alkaloid (C) fraction was inactive at doses ranging from 6.35 to 50.0 mg./kg. i.p., whereas leurosine was highly active against the same neoplasm at doses ranging from 0.375 to 3.0 mg./kg. i.p. (4).

[1] Woelm, activity grade III.

DISCUSSION

The anomalous isolation of an active antitumor alkaloid from a crude alkaloid fraction of *C. lanceus* prompted the thorough investigation of all alkaloid fractions from this plant, whether they are active or inactive against the P-1534 leukemia. The only speculation that can be offered at this time for this phenomenon is that the alkaloid (C) fraction contains, in addition to leurosine, a material that displays a delayed toxicity. Animals receiving the extract may be adversely affected only at about the time that the leukemic control group of animals die, *i.e.*, about 12–15 days from the time of infection. If this is indeed the reason, it might be profitable to subfractionate each crude alkaloid fraction and have each of these evaluated if the original fraction gives a negative antitumor test.

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Cycloheptaamylose–Barbiturate Inclusion Complexes: Solubility and Circular Dichroism Studies

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Abstract □ The interaction of cycloheptaamylose with some barbiturates was examined by solubility analysis and by circular dichroism. Complexation by inclusion of the barbiturate within the cavity of cycloheptaamylose leads to: (*a*) an enhancement in the aqueous solubility of the barbiturate, and (*b*) extrinsic Cotton effects in the circular dichroism spectra. Measurements from both methods yield formation constants for the 1:1 interactions. The relative strength of interaction with cycloheptaamylose is of the following order: phenobarbital > pentobarbital > amobarbital > barbital.

Keyphrases □ Cycloheptaamylose–barbiturate inclusion complexes—solubility and circular dichroism studies □ Barbiturate–cycloheptaamylose inclusion complexes—solubility and circular dichroism studies □ Circular dichroism—analysis of cycloheptaamylose–barbiturate inclusion complexes □ Interactions—cycloheptaamylose with phenobarbital, pentobarbital, amobarbital, and barbital □ Complexes, inclusion—cycloheptaamylose–barbiturate, solubility and circular dichroism studies

Cycloamyloses (cyclodextrins) are known to form inclusion complexes with drug molecules of a variety of structure types (1–5). The complexation of some barbituric acid derivatives with cycloheptaamylose was the subject of a preliminary report from this laboratory (6). Cycloheptaamylose was shown to complex with barbiturates by solubility and proton magnetic resonance techniques; the corresponding formation constants were obtained from solubility data. In the present report, details of the solubility measurements are given.

This paper is also concerned with an examination of the complexes by circular dichroism (CD), a technique that has been suggested (7–9) but not extensively applied before to cyclodextrin complexes. Inclusion of the barbiturate within the cavity of cycloheptaamylose results in extrinsic optical activity. The induced Cotton effects can be quantitatively treated to yield formation constants.

EXPERIMENTAL

Materials—Recrystallized amobarbital, m.p. 156–158°, recrystallized barbital, m.p. 190–192°, recrystallized pentobarbital, m.p. 129–130°, and phenobarbital USP were used as received. Cyclohepta-