# Modern Approaches for Selecting Biologically Active Plants I: CNS Depressants

N. R. FARNSWORTH[x], J. P. BEDERKA, Jr., and M. MOSES

**Abstract** □ Based on literature reports and other arbitrarily assigned criteria, a method is suggested with which one can select plants with high theoretical probability for CNS depressant activity. Their extracts experimentally demonstrate this activity. The method was tested by selecting 11 high scoring plants and 16 low scoring plants from a list of 575 plants which theoretically should contain CNS depressant principles. These 27 plants were screened for CNS depressant activity utilizing the activity cage. A statistically significant difference was found between plants selected from the top of the list and those selected from the bottom of the list.

**Keyphrases** □ Medicinal plants—novel approach for selecting plants with CNS activity □ Phytological screening—novel approach for selecting biologically active plants, CNS activity □ Screening, biologically active plants—novel approach for selection using literature and assigned criteria

Most useful drugs obtained from plants have been discovered following investigations initiated on the basis of their medicinal folkloric descriptions. One can cite digitoxin *(Digitalis purpurea)*, reserpine *(Rauvolfia serpentina)*, ergonovine and ergotamine *(Claviceps purpurea)*, khellin *(Ammi visnaga)*, atropine *(Atropa belladonna)*, and scopolamine *(Datura stramonium)* as major examples. On the other hand, many investigations of plants that were designed on the basis of their folkloric reputations have not yielded biologically active compounds; indeed, extracts of many such plants could not be shown to have the folkloric biological activity when evaluated in laboratory animals. Therefore, many investigators discount altogether the folkloric approach as a means of discovering new biologically active entities.

A second approach to discovering plants having useful biologically active agents has been to screen large numbers of randomly selected plants for one or more types of biological activity (1–5). The clinically important antitumor agents vincristine (6) and vinblastine (6) were discovered in this way. A number of other promising antitumor agents have likewise been discovered through this approach, including acronycine (7), camptothecin (8), thalicarpine (9), fagaronine (10), *Acer*-saponin-P (11), tetrandrine (12), maytansine (13), and bruceantin (14). But critics of this mass screening approach point out that it is expensive and unscientific.

It appeared that some other means should be available for selecting a few candidates for evaluation, for a specific and desired type of biological activity, from the more than 750,000 estimated species of higher plants. About a 3-year period from the scientific literature together with an arbitrary scoring system was used to rank a list of plants that might be expected to exert a sedative effect in laboratory animals. This ranking was followed by the testing of extracts of plants from this list for central nervous system (CNS) depressant activity in mice to evaluate the effectiveness of the method.

**Table I**—Criteria for Selection of Potential Sedative-Containing Plants

| Points Assigned | Criteria |
|---|---|
| 10 | Plant reported in the literature as having some degree of CNS depressant activity as a result of biological evaluation in laboratory animals |
| 6 | Plant for which credible folkloric references were found in the literature, in which some type of sedative activity was mentioned for the plant |
| 5 | Plant taxonomically related to a group of plants that was well known to yield effective sedative principles (at either the family or genus level) |
| 4 | Plant for which literature reports indicated that alkaloids were present in the plant or had been isolated from the plant [Two recent compilations covering the world literature on this subject were consulted (16, 17)] |
| 3 | Plant classified in a group that had received little or no chemical or biological investigation, as evidenced from literature files or general knowledge |
| 2 | Plant for which two or more species of the genus in question had been assigned both 10 and 6 points as indicated above |
| −5 | Plant for which literature sources directly indicated any type of toxicity ascribed to the plant, its extracts, or isolated compounds, either experimentally or on the basis of folklore |

## EXPERIMENTAL

**Plant Selection**—A literature search[1] from the years 1970–1972 and sporadically prior to 1970 revealed a total of 575 plants that had either been reported as folkloric sedative remedies or whose extracts had been reported to show sedative or CNS depressant activity when tested in laboratory animals. Each of the 575 plants was assigned points according to the arbitrarily appointed criteria presented in Table I. By using this system, a maximum of 30 points or a minimum of −5 points could be assigned to any given plant. It was found that 75 plants were assigned 15 or more points, which was an arbitrarily selected cut-off point for further consideration of plants in this study. By inspection, a number of plants were eliminated from this list of 75 on the following grounds. Plants that were well known for their sedative or CNS depressant properties or for which the active principle(s) was (were) known were removed from the list, *i.e.*, *R. serpentina*, *Cannabis sativa*, and *Papaver somniferum*. Also, plants that contained uncharacterized alkaloids on the basis of literature reports and that were in such taxa as the Leguminosae were removed from the list. The rationale here was that many Leguminosae alkaloids are of the pyrrolizidine type, which are usually very toxic. Similarly, plants containing coumarins, a group of compounds usually toxic to the liver, were discounted.

Plants were eliminated from the list of 75 for the reasons given until a final list of 20 plants was achieved. It was hoped to acquire samples of all 20 plants and to evaluate them in animals for

[1] *Chemical Abstracts* and *Biological Abstracts*.

**Table II**—$ED_{50}$ Values for Plant Extracts from the Upper 20 Selections

| Plant | $ED_{50}$, mg/kg ip |
|---|---|
| SP-01 | 28.0 |
| SP-02 | 56.0 |
| SP-03 | 66.0 |
| SP-04 | 70.0 |
| SP-05 | 70.0 |
| SP-06 | 72.0 |
| SP-07 | 76.0 |
| SP-08 | 110.0 |
| SP-09 | 118.0 |
| SP-10 | 120.0 |
| SP-11 | 250.0 |
| Sodium pentobarbital | 27.0 |

CNS depressant activity. However, it was only possible to acquire 11 of the 20 plants, but this number was considered a representative sample.

For comparative purposes, and to test the validity of the criteria used for the selection of plants, an attempt was made to obtain the plants with the lowest number of points on the list of 575. It was possible to obtain 16 of the lowest scoring 20 plants.

The arbitrary criterion assigning four points to plants containing alkaloids seemed to bear a distinct relationship to the 575 plants initially selected. Seventy-five percent of the 575 plants selected were reported in the literature to contain alkaloids, but the normal distribution of alkaloids in plants on a random-selection basis is only 5–10% (15). Thus, alkaloids in general could be considered as a type of marker for CNS depressant activity.

**Preparation of Extracts for Testing**—Each plant sample was milled to a coarse powder, and 200 g was added to 1.0 liter of boiling distilled water. The mixture was allowed to cool to room temperature and then was filtered through a Büchner funnel. The filtrate was then frozen and lyophilized. All extracts were prepared in an identical manner, and subsequent biological tests could be compared on a weight basis. All extracts were soluble in water at the concentrations tested. Immediately prior to use, the dry powder was dissolved in a convenient volume of distilled water. This method of sample preparation was utilized since one criterion employed for plant selection was that the plant had been described in the literature as having been used as a folkloric remedy to induce sleep, to act as a tranquilizer or narcotic, or for some other type of CNS depressant activity. The method of preparation of test extracts was similar to the method that would have been used by natives, or by the laity, in preparing such an infusion, both in procedure and in amounts used.

**Evaluation for CNS Depressant Activity**—Pharmacological evaluation of the potential sedative activity of the plant extracts was determined in female albino mice[2] weighing 16–25 g. Mice were initially divided into five groups of five animals each for preliminary screening of the extracts. All extracts were administered in graded doses of 1–1600 mg/kg ip in amounts not exceeding 0.5 ml/mouse. Different groups of control animals received intraperitoneal injections of water in amounts that varied between 0.1 and 0.5 ml. No effect of volume of injection on gross motor activity was found in the controls. Sodium pentobarbital was used as the sedative reference compound.

Four- or five-dose levels of each extract or of sodium pentobarbital were tested, and dose–response curves were determined.

Two naive control groups were employed on each day (morning and afternoon), and the data from the extract-treated groups were compared with their respective morning and afternoon control data. Naive mice were used since previous exposure to the counting chamber and retesting showed approximately a 50% reduction in activity. Each point on the final dose–response curves represented a mean of either duplicate or triplicate consecutive determinations with five mice in each treatment group. The percent decrease in motor activity in most cases ranged from 20 to 80% of controls.

Spontaneous motor activity was measured with an activity cage[3] equipped with photoelectric cells for recording gross movements of the mice by a mechanical counter. Counting was performed for 30 min following injection of the test solutions. The data obtained were plotted graphically as the percent decrease in motor activity against the logarithms of the doses used. The $ED_{50}$ values[4] for sodium pentobarbital and each plant extract were obtained from graphical extrapolation of the 50% point on the ordinate to the regression line and then to the corresponding dose on the abscissa.

The mean $ED_{50}$ values, calculated for the 11 plants selected from the top 20 plants on the list of 575, are presented in Table II. Similar data for the 16 plants selected from the lower 20 of the 575 are presented in Table III.

**Table III**—$ED_{50}$ Values for Plant Extracts from the Lower 20 Selections

| Plant | $ED_{50}$, mg/kg ip |
|---|---|
| SP-12 | 1.8 |
| SP-13 | 9.0 |
| SP-14 | 38.0 |
| SP-15 | 67.0 |
| SP-16 | 92.0 |
| SP-17 | 94.0 |
| SP-18 | 138.0 |
| SP-19 | 205.0 |
| SP-20 | 230.0 |
| SP-21 | 220.0 |
| SP-22 | 370.0 |
| SP-23 | 520.0 |
| SP-24 | 610.0 |
| SP-25 | 665.0 |
| SP-26 | 1000.0 |
| SP-27 | 1360.0 |
| Sodium pentobarbital | 27.0 |

## DISCUSSION

The purpose of this study was to test a hypothesis that a few plants could be selected from many that have either a folkloric reputation as sedatives or for which extracts may have shown some sedative activity in laboratory animals. The criteria presented (Table I) are in part highly subjective and rely to some degree on the depth of knowledge of the natural product literature possessed by the person assigning the scores; nevertheless, the results seem to establish that the method has some merit. The names of specific plants are not listed since they are irrelevant to what is being reported, *i.e.*, a method for selecting plants having a high degree of sedative activity.

The method has been shown to be effective since the mean $ED_{50}$ values of the two groups of extracts were significantly different. The $ED_{50}$ of the 11 tested plants from the 20 plants that scored highest was 87 mg/kg, with a standard error of ±18 mg/kg. In contrast, for the 16 evaluated plants from the 20 lowest scoring plants, the $ED_{50}$ was 351 mg/kg, with a standard error of ±97.5 mg/kg. The difference between these two mean doses was significant at the $p = 0.02$ level (Student $t$ comparison).

The accurate results for selecting sedative plants were achieved by utilizing the equivalent of only about 3 years of the available scientific literature. If a larger data base had been employed, it is reasonable to assume that the results might have been improved.

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[2] Charles River (SCR).
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[4] The $ED_{50}$ is equivalent to that dose of the extract required to decrease spontaneous motor activity 50%.

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# Mechanisms of Hydrolysis of Salicylanilide *N*-Methylcarbamates

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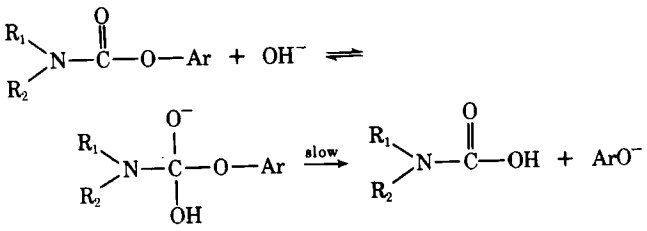
**Abstract** □ The facile hydroxide-ion-catalyzed hydrolysis of the carbamoyl group in salicylanilide *N*-methylcarbamate (I) and analogs unsubstituted on the anilide nitrogen proceeds by a reaction mechanism involving anchimeric assistance by the *o*-carboxanilide group. Substitution of an alkyl or aryl group for the anilide proton greatly reduces the hydrolysis rate; the reaction rate constant for I is ~7 × $10^2$ times that for *N*-methylsalicylanilide *N*-methylcarbamate (II). To characterize the mechanism for II, comparative parameters of activation were determined for I and II. The entropy of activation ($\Delta S\ddagger$) for II was 8.6 eu more negative than that for I, suggesting steric hindrance in the transition state for II. The observed reaction rate constant for II was consistent with estimates based on the electronic *ortho*-substituent effect of the carboxanilide group. It is concluded that II is hydrolyzed *via* the established mechanism for simple aryl *N*-monosubstituted carbamates (rapid proton extraction followed by a rate-determining conversion of the carbamate anion to an isocyanate) rather than by the mechanism followed by I.

**Keyphrases** □ Salicylanilide *N*-methylcarbamates—mechanisms of hydrolysis □ Hydrolysis mechanisms—salicylanilide *N*-methylcarbamates □ Carbamoyl group hydrolysis—mechanisms of salicylanilide *N*-methylcarbamate hydrolysis

A growing body of literature (1–7) has established a duality of mechanism for the alkaline hydrolysis of aryl carbamates. In the absence of a proton on the nitrogen atom (*N,N*-disubstituted carbamates), the reaction follows Scheme I, characteristic of simple ester hydrolysis. However, for compounds containing a proton on the nitrogen atom (*N*-monosubstituted carbamates), hydrolysis occurs *via* Scheme II (the ElcB mechanism).

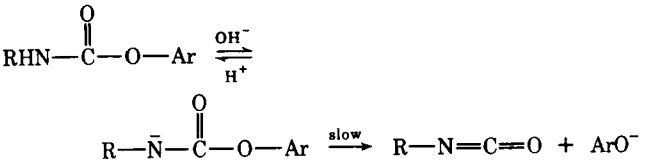
Reports from these laboratories described the kinetics of hydrolysis of a number of aryl carbamates possessing anti-inflammatory activity (8–10). Hsi *et al.* (9) showed that the extremely rapid hydroxide-ion-catalyzed hydrolysis of the carbamoyl group of



*Scheme I*

salicylanilide *N*-methylcarbamate (I) and a number of analogs with *para*-substituents in the phenyl ring of the anilide moiety involved participation by the neighboring *o*-carboxanilide group. Reaction rate constants, $k_{OH^-}$, were logarithmically related to the Hammett $\sigma$-constants ($\rho$ = 0.46 at 37°). A special case of the mechanism shown in Scheme II, involving rapid proton extraction from the carbamate nitrogen followed by a rate-limiting intramolecular cyclization and subsequent ring opening and fragmentation, was indicated (Scheme III).

Substitution of an alkyl or aryl group for the proton on the anilide nitrogen of I produces a dramatic reduction in the carbamate hydrolysis rate (9). Reaction rate constants for *N*-methylsalicylanilide *N*-methylcarbamate (II) and *N*-phenylsalicylanilide *N*-methylcarbamate (III) are nearly identical and only about 15 times that for phenyl *N*-methylcarbamate



*Scheme II*