Occurrence of a Weak Anti-inflammatory Substance in *Simaba cedron* Seed

By E. ROY HAMMARLUND

Ethanol and aqueous extracts of *Simaba cedron* seeds contain a weak, nonspecific, and relatively toxic anti-inflammatory substance.

It was suggested that *Simaba cedron* seed, *Simaroubaceae*, possibly contained an anti-inflammatory or antiarthritic agent because a 5% extract of the powdered seeds in 70% ethanol had been used orally in Denmark for rheumatoid arthritis with some apparent success (1).

Previous investigations on the plant have been few and the results have been contradictory. Nauck and Picado (2) reported limited success with injections of an alcoholic extract of cedron seed as a treatment for malaria, whereas Spencer, *et al.* (3), found no antimalarial activity present. Gauckler (4) described the use of the cotyledons as a remedy for snakebite and fever, whereas Bonsmann (5) reported that an extract of cedron had no antisnakebite activity. Krebs and Rüber (6) isolated cedrin, a nonalkaloid or glycosidal bitter principle, and reported that it is probably a partially hydrated naphthalene derivative similar to santonin. However, no tests were made for any biological activity. Polonsky (7) isolated two nonalkaloidal cedron fractions, cedronine and cedronyline, but likewise did not test for biological activity. Moreover, Wall, *et al.* (8), in their numerous surveys of plants for the presence of steroidal sapogenins did not investigate *Simaba cedron*.

The object of this initial investigation was to make several crude extracts of the powdered dried cedron seeds by employing various solvents and to biologically screen these extracts on rats for the possible presence of any anti-inflammatory principle. The finding of any appreciable amount of activity would then have been followed by an intensive search for, and the isolation of, the particular biologically active ingredient. However, none of the extracts yielded a substance sufficiently active to warrant further and more complete investigation. Nevertheless, because there may be some value in an essentially negative report, the experimental procedures and findings of this study are here reported very briefly.

EXPERIMENTAL

Extraction Procedure.—Dried seeds which had been collected in South America were verified by botanists of S. B. Penick and Co. and E. R. Squibb and Co. The seeds were finely powdered in a grinder and this powder was used for all extractions. Approximately 500 Gm. of the powdered seeds were used for each of the individual extractions by the following solvents: petroleum ether, anhydrous ethyl ether, ethanol, and water. Three or four repeated extractions were made with each solvent on a separate batch of seeds using a high-speed blender at room temperature. No attempt was made to obtain a complete extraction with any solvent. The solvents were evaporated in a rotary film evaporator without heat and the following amounts of extractives were obtained: with petroleum ether, 500 Gm. of powdered seeds yielded 36.6 Gm. of a yellow oil; with anhydrous ethyl ether, 500 Gm. of powdered seeds yielded 48.8 Gm. of a viscous yellow oil which contained a small amount of white solids; with ethanol, 500 Gm. of powdered seeds yielded 27.1 Gm. of a sticky dark yellow solid; with distilled water, 400 Gm. of powdered seeds yielded 37.8 Gm. of a dried yellow solid which could be powdered.

These extracts were dried in a vacuum desiccator and the vials were tightly stoppered under nitrogen. The samples were sent to the Squibb Institute for Medical Research for routine screening for the presence of anti-inflammatory activity.

Biological Screening Tests and Results.—The four extracts were assayed in both intact and adrenalectomized male rats implanted with cotton pellets. Results of these studies indicate that the petroleum ether soluble and anhydrous ethyl ether soluble extracts were inactive even at daily subcutaneous doses of 10 mg. per animal. The ethanol soluble extract was lethal at 10 mg. in the adrenalectomized animal. This ethanol extract induced a slight anti-inflammatory effect in the intact rat at daily doses of 0.4 mg. and 2 mg. The water soluble extract was lethal in the intact rat at a 10-mg. dose and had a weak anti-inflammatory effect at a 4-mg. daily dose, but was inactive at lower doses. In additional studies in intact animals the water soluble extract partially prevented (28%) the hind limb edema induced by the parenteral administration of dextran.

DISCUSSION

The results indicate that the extracts of the seeds are highly toxic and have only a weak, nonspecific anti-inflammatory activity. A large number of toxic or irritating substances such as turpentine could produce similar effects in these same tests. Therefore, there is little justification for continuing the search for an anti-inflammatory agent in this plant. No attempt was made to identify the slightly active substance.

Since the aqueous solutions of the powdered seeds foamed so copiously during the extraction and evaporation, it was suspected that there were probably saponins, sapogenins, or protein material present. Using a DuNouy tensiometer, a 1% aqueous solution of the aqueous extract was found to lower the surface tension of distilled water a total of 28.1 dynes/cm. at room temperature. Furthermore, in order to find out if this aqueous solution was strongly hemolytic to red blood cells, two drops of fresh human capillary blood were added to flasks containing 25 ml. of 0.1%, 1.0%, and 5.0% aqueous solutions of the extract each in 0.6%, 0.75%, and 0.9% sodium chloride solutions. The solutions were stoppered,

Received May 21, 1962, from the College of Pharmacy, University of Washington, Seattle.
Accepted for publication June 13, 1962.
The author is indebted to the Squibb Institute for Medical Research for carrying out the biological screening tests.

mixed by inversion, and allowed to stand one hour at room temperature and for an additional 24 hours at approximately 5°. None of the solutions caused any hemolysis of the erythrocytes by the end of the 25 hours. This indicates that the saponin content is not great and the extract is not strongly hemolytic.

The powdered seeds were further investigated for the presence of alkaloids by employing the routine acidic-aqueous and alkaline-chloroform partition extraction procedure twice and then testing the aqueous solution with Mayer's reagent and, likewise, the chloroform solution on a spot plate with Erdmann's, Froehde's, and Mandelin's reagents. All tests were negative, which indicates the absence of alkaloids in cedron seeds.

SUMMARY

1. *Simaba cedron* seeds contain a weak, nonspecific, and relatively toxic anti-inflammatory substance.

2. An aqueous extract of the seeds is nonhemolytic to erythrocytes and does not contain alkaloids.

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Application of Solvent Extraction and Anion-Exchange Chromatography to the Determination of Sodium Pantothenate in Pharmaceutical Products

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A spectrophotometric method, developed for calcium *d*-pantothenate, was demonstrated to be equally applicable to the sodium salt.

A CHEMICAL method for the determination of calcium *d*-pantothenate in pharmaceutical products was recently developed in this laboratory (1). The method was based in part on an extraction with benzyl alcohol in the presence of a salting-out agent and on an anion exchange chromatography. Since different salts were shown to affect the partition coefficient of the pantothenate in the solvent extraction, it was necessary to investigate the applicability of the method to sodium *d*-pantothenate which is used in some pharmaceutical products. The possibility of using the calcium salt as a reference standard in the determination of the sodium form was also studied by comparing the relative absorbance produced by the reaction product of the two salts.

EXPERIMENTAL

Preparation of Sodium Pantothenate.—A 5.0-Gm. quantity of calcium *d*-pantothenate was dissolved in a minimum volume of distilled water and 21.5 ml. of a 5% solution of sodium carbonate was added with stirring. The solution was let stand overnight in a refrigerator, then filtered on a No. 1 Whatman. The precipitate was washed with about 350 ml. distilled water. The combined filtrate and washings was adjusted to pH 6.0, and made up to 500 ml. with distilled water.

Received June 11, 1962, from the Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada.
Accepted for publication June 26, 1962.
Helpful discussions concerning this paper by Dr. J. A. Campbell and statistical assistance of Mr. J. Malcolm Airth are gratefully acknowledged.

Recovery Test.—Amounts of 1.235, 2.470, and 4.940 ml. of the prepared solution were each added to 25 ml. of a liquid multivitamin preparation. These amounts were equivalent to 0.5, 1.0, and 2.0 mg. of sodium *d*-pantothenate per ml. of the liquid sample. The determinations were made in duplicate on aliquots of 10 ml. of the solutions after being diluted to a 100 ml. with distilled water. Calcium *d*-pantothenate was the reference standard and a factor of 1.0124 was used for the conversion of calcium *d*-pantothenate to its equivalent of the sodium salt.

RESULTS AND DISCUSSION

To compare the behavior of the two salts, 5 mg. of the calcium salt and an equivalent weight of the prepared sodium salt were subjected to the procedure (1). The assays were conducted in three replicates on different days. The results, presented in Table I, indicated that although there was significant variation between assays for the sodium salt there was no significant difference on the average between the absorbance of the reaction products of the two salts. No apparent destruction of the pantothenate radical due to hydrolysis was observed. Since it was shown previously that the presence of amino acids did not significantly affect the final absorbance readings (1), and that an equal response of both forms of the pantothenate was obtained in this experiment, it was concluded that β-alanine was not formed before the pantothenate was subjected to the complete analytical procedure.

The results of the recovery test of sodium *d*-