Bacteriostatic Property of *Aloe vera*

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Freeze-dried juice obtained from *Aloe vera* and heated for 15 minutes at 80° inhibited several test microorganisms.

SPECIES OF *Aloe* have had a long history as drug plants. This was pointed out by Morton (1) in a recent comprehensive review of *Aloe* from the standpoint of folk use and commercial exploitation. Fly and Kiem (2) recently carried out an invesigation to ascertain whether *Aloe vera* exhibits antimicrobial activity. They reported that macerates of the central gelatinous portion, of the green vascular portion, and of the complete leaf of *A. vera* did not exhibit, within the limits of their experiment, antimicrobial effect against *Staphylococcus aureus* and *Escherichia coli*.

Since it has been established definitely in this laboratory that the fresh juice of *A. vera* L. contains a principle(s) which is inhibitory to certain microorganisms, the results are reported in this note.

EXPERIMENTAL

Leaves of *A. vera* L. were cut at the base and stood upright so that the juice could drain from the leaves into receptacles. If tested immediately, the fresh juice exhibited a marked zone of inhibition of *S. aureus* 209. However, the principle responsible for the inhibitory activity was found to be unstable. Preservatives as sodium bisulfite, sodium benzoate, and methyl paraben were ineffective; however, the principle could be temporarily preserved by refrigeration and preserved for an even longer period by heating the juice for 15 minutes at 80°. In all instances the juice would gradually turn dark. Once the juice became dark, the inhibitory property was lost. If the juice that had been heated for 15 minutes at 80° was freeze-dried, a buff-colored product resulted which was stable.

A solution of the freeze-dried juice (20 mg./ml. of distilled water) was tested by the agar diffusion technique for bacteriostatic activity against the following organisms: *S. auerus* 209, *E. coli*, *Streptococcus pyogenes*, *Corynebacterium xerose*, *Shigella paradysenteriae*, *Salmonella typhosa*, *Salmonella schottmuelleri*, and *Salmonella paratyphi*. After a period of incubation at 37° for 24 hours, significant inhibition of growth was evident on plates inoculated with *S. aureus* 209, *S. pyrogenes*, *C. xerose*, and *S. paratyphi*.

The whole leaf minus the juice, the leaf mesophyll, and the leaf epidermis were each separately freeze-dried and successively extracted with petroleum ether (b.p. 30–60°), ether, chloroform, ethanol, and distilled water. None of the extracts exhibited inhibitory activity against the test organisms.

Since the juice of *Aloe* is known to contain anthraquinone-type compounds, aloe-emodin, emodin, and chrysophanic acid were tested for inhibition of *S. aureus* 209. The results were negative.

CONCLUSIONS

While the freeze-dried whole leaf minus the juice, the leaf mesophyll, and the leaf epidermis of *A. vera* L. did not exhibit bacteriostatic properties, the freeze-dried juice previously heated for 15 minutes at 80° did inhibit *S. aureus* 209, *S. pyogenes*, *C. xerose*, and *S. paratyphi* using the agar diffusion test method.

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Communications

Determination of Isomeric Purity of Dextroamphetamine in Tablets and Capsules

Sir:

The tablet form of dextroamphetamine sulfate is widely used, has been official since the 15th revision of the "United States Pharmacopeia," and is produced by a large number of pharmaceutical companies. Enforcement work has brought to light several examples of adulterated products in which the *dextro* isomer has been replaced completely or in part by the less expensive racemate (1, 2). Nevertheless, the U.S.P. does not have a method to ascertain the isomeric purity of the drug present in the tablets. Because of the low specific rotation of dextroamphetamine sulfate (+23.5°) and the volatility of the base, certain derivatives are more suitable for determination of the optical activity. Acetylamphetamine has been particularly useful for this purpose. It can be prepared easily in good yield and its specific rotation (in chloroform) is about twice that of dextroamphetamine sulfate and of opposite sign (−44.0°). The main problem is the extraction and purification of the amine from the tablets prior to acetylation. Capsules of dextroamphetamine sulfate, especially those having sustained-release action, also present difficulties in the isolation of the base. In 1953, a procedure was de-

TABLE I.—ISOMERIC PURITY OF COMMERCIAL DEXTROAMPHETAMINE SULFATE TABLETS AND CAPSULES

Mfr.	Product, mg.	Specific Rotation 1	Specific Rotation 2	Isomeric Purity, Av., %
A	Tablets, 5	−37.8°	−38.3°	93.2
B	Tablets, 5	−40.4°	−39.1°	95.2
C	Tablets, 5	−39.2°	−38.7°	94.3
D	Tablets, 10	−43.2°	−42.3°	98.6
E	Sustained-release capsules, 10	−38.7°	−39.5°	94.4

veloped by Welsh (1) for determination of the isomeric purity of dextroamphetamine in tablets based on the specific rotation of the acetyl derivative. This method, adopted by the Association of Official Agricultural Chemists (3), uses the titrated solution obtained after completion of the quantitative determination of dextroamphetamine sulfate as described in the official monograph. It has been recognized for some time that the assay of dextroamphetamine tablets in U.S.P. XVI is unsatisfactory and is, in fact, completely unsuitable for several of the preparations on the market (2). It will be replaced in U.S.P. XVII by a spectrophotometric method which utilizes partition chromatography for isolation and purification of the drug (4). However, this is a micromethod requiring only 5 mg. of dextroamphetamine sulfate, a quantity not enough for determination of the specific rotation. The difficulties of extracting a sufficient amount of dextroamphetamine from tablets and capsules have been eliminated by means of chromatography on aluminum oxide. The coloring agents and other excipients remain on the column, producing a clear colorless solution of dextroamphetamine which can be used for preparation of the acetyl derivative.

Procedure.—A pledget of fine glass wool is packed with a packing rod in the base of a chromatographic tube, 25 × 200 mm., and 5 Gm. of basic chromatographic alumina[1] is added to the tube and compressed firmly to a uniform mass. Dextroamphetamine sulfate tablets (or the contents of capsules), equivalent to about 130 mg. of dextroamphetamine sulfate, are reduced to a fine powder and mixed thoroughly in a mortar with 5 Gm. of basic chromatographic alumina.[1] After addition of 1.0 ml. of methanol and 0.5 ml. of stronger ammonia T. S., the mixture is triturated and, when uniform, transferred without delay to the chromatographic column and compressed with a packing rod. The mortar and pestle are wiped with a little glass wool which is inserted into the tube on top of the column. A 125-ml. separator containing 35 ml. of 0.1 *N* sulfuric acid is placed under the column as a receiver, and 60 ml. of chloroform is passed through the column. The separator is shaken vigorously for 1 to 2 minutes, the layers are allowed to separate, and the chloroform discarded. To the liquid in the separator is added 2.5 Gm. of sodium bicarbonate, and the mixture is swirled until most of the bicarbonate has dissolved. With a 1-ml. syringe, 1.0 ml. of acetic anhydride is injected rapidly into the contents of the separator. The separator is stoppered immediately and shaken vigorously until the evolution of carbon dioxide has ceased. The pressure is released as necessary through the stopcock. The solution is allowed to stand for 5 minutes, then extracted once with 50 ml. of chloroform. The chloroform extract is filtered through a pledget of cotton into a 100-ml. beaker. The cotton is rinsed with a little chloroform, and the solution evaporated to dryness on a steam bath in a current of air or nitrogen. The residue is heated and triturated with a glass rod until the odor of chloroform can be detected no longer. As the residue cools, it is induced to crystallize, powdered finely, and heated to 80° for 30 minutes.

After cooling in a desiccator, about 100 mg. of the crystalline acetylamphetamine, accurately weighed, is transferred to a 5-ml. volumetric flask and dissolved in chloroform. The optical rotation is determined by means of a 200-mm. semimicro polarimeter tube and the specific rotation calculated.

The procedure has been used for a number of commercial samples of dextroamphetamine sulfate tablets and capsules. The results in Table I indicate that the method has good reproducibility.

Optically pure dextroamphetamine sulfate has a specific rotation of +23.5° (1). On this basis, the official tolerance limits of +20 to +23.5° correspond to 92.6 to 100.0% of the *dextro* isomer. Dextroamphetamine sulfate of 92.6% isomeric purity would yield an acetyl derivative with a specific rotation of −37.5°.

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[1] Aluminum oxide, Merck (Darmstadt), standardized according to Brockmann, was used in this work.

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