Chemical Examination of *Clerodendron serratum.* Isolation and Characterization of D-Mannitol

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D-Mannitol has been isolated from the root bark of *Clerodendron serratum* in a yield of 10.9 per cent and identified by mixed melting point, by analysis, chromatographic behavior, and by preparation of hexa-acetyl, hexanitro, hexabenzoyl derivatives, and benzaldehyde condensation product.

CLERODENDRON SERRATUM Spreng (N.O. *verbinaceae*) (1), popularly known in India as Bharangi, is a small flowering shrub found more or less throughout India, Ceylon, and the Malay peninsula. Various parts of the plant are credited in the Indian system of medicine with usefulness in bronchitis, asthma, eczema, catarrhal affections of lungs, febrile, and other manifestations. Aqueous extracts of the root bark of the plant have been found by Sachdev *et al.* (2) to block selectively the histamine-induced responses in isolated guinea pig ileum and on blood pressure in anesthetized dogs. The antihistaminic effects have been confirmed by Gupta *et al.* (3) in this department. Although it was considered by Sachdev *et al.* (2) that the active material could be precipitated from the aqueous extracts by addition of ethanol, a systematic chemical examination of the plant for its constituents has not been described before. During the course of fractionation of the aqueous extracts for isolating the antihistaminic principles, it has been found that the root bark contains a considerable amount (10.9%) of D-mannitol, whose isolation and identification are presently reported.

EXPERIMENTAL

Isolation of D-Mannitol

The dried root bark, freed from outer green skin and foreign matter, was reduced to about No. 20 mesh powder (500 Gm.) and was extracted with four changes of distilled water by boiling for 15 min. each. After filtering through muslin, the combined extract (3.5 L.) was allowed to stand for 3 days in an ice chest to allow complete deposition of the suspended matter. The clear supernatant was diluted 2.5 times with water and boiled briefly with active charcoal (108 Gm.). On concentrating the colorless filtrate to about 150 ml. and cooling, colorless crystalline needles of crude D-mannitol separated slowly over a number of days. The crystals were, however, collected after 3 days and further purified by passing, in aqueous solution, through a column of ion-exchange resin[1] (H) to remove traces of calcium salts that were found to be present. Yield, about 1%.

Better yield (10.9%) of D-mannitol was obtained by soxhleting the root powder with 95% ethanol for several hours, most of the extracted D-mannitol crystallizing during the extraction process. After final purification by treatment with the resin[1] and crystallization from hot 95% ethanol, the substance melted at 166° (corrected).

Anal.—Calcd. for $C_6H_{14}O_6$: C, 39.55; H, 7.74; mol. wt., 182. Found: C, 39.67; H, 7.67; mol. wt. (ebullioscopic), 188.9.

Characterization as D-Mannitol

Solubility—The isolated substance was very slightly soluble in hot 95% ethanol and insoluble in ether, chloroform, petroleum ether, benzene, and ethyl acetate. It was highly soluble in water, the solution being neutral to litmus.

Reactions—It did not reveal the presence of aromatic character or the aldehydic, ketonic, carboxyl, and phenolic groups or unsaturation. The substance, however, changed the color of ceric ammonium nitrate reagent to orange red and discharged the pink color of 1% borax solution due to phenolphthalein, the color reappearing on warming. Like D-mannitol, it prevented the precipitation of $Fe(OH)_3$ on addition of NaOH solution to a solution of $FeCl_3$ and gave a flocculent precipitate with an ammoniacal solution of copper sulfate. Its melting point on admixture with a pure authentic sample of D-mannitol was undepressed.

Optical Rotation—An aqueous solution (10%) of the substance gave no evidence of optical activity while 10% solution in 0.2 N borax gave $[\alpha]_D^{25}$ +30° after 3 hr.

Chromatographic Identification—The substance was found to be chromatographically homogeneous with the authentic sample on Whatman No. 1 filter paper using butanol–ethanol–water solvent (4:1.1:1.7). Its mobility was impeded on borated chromatographic strips, which is characteristic of D-mannitol.

Derivatives—The substance gave hexa-acetyl, hexanitro, hexabenzoyl derivatives, and a benzaldehyde condensation product. The properties of these derivatives agree with those given in literature for such derivatives of D-mannitol.

Hexa-acetate—Obtained readily by treatment with acetic anhydride and fused sodium acetate, m.p. 122° (corrected).

Anal.—Acetyl value: Calcd. for $C_6H_8O_6$ (Ac)$_6$: 59.47. Found: 61.5.

Hexanitrate—Prepared by treating this substance (1 part) dissolved in concentrated HNO_3 (5 parts) with concentrated H_2SO_4 (10 parts) in cold, m.p. 112° (corrected).

Anal.—Calcd. for $C_6H_8N_6O_{18}$: N, 18.59. Found: N, 18.50.

Hexabenzoate—Prepared by treating the substance with benzoyl chloride in presence of pyridine, m.p. 148° (corrected).

Benzaldehyde Condensation Product—The condensation (4) of D-mannitol (100 mg.) with benzaldehyde (200 mg.) was more satisfactorily achieved using phosphorus pentoxide (100 mg.) or concentrated sulfuric acid (2 drops), than with concentrated HCl. After shaking the mixture for 15 min., the product was washed with dilute sodium carbonate solution, water, and ether and crystallized from ethanol. The condensation product melted at 220° (corrected) (*cf.* m.p. 223–224°) (4).

REFERENCES

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Observations Concerning the Correlation of *In Vitro* Sulfonamide Activity with pKa and the Hammett Values

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Linear correlations of the *in vitro* bacteriostatic activity of sulfonamides with pKa provide little evidence in support of there being a definite pKa which a sulfonamide must possess in order to exhibit maximum activity.

BELL AND ROBLIN (1) first reported a correlation between the *in vitro* bacteriostatic activity of a series of sulfonamides and their pKa values. They summarized their proposition in the statement: "the more negative the SO_2 group of a sulfonamide type compound, the greater the bacteriostatic activity of the compound . . . . .; the correlation between acid dissociation (pKa) and the activity is shown to be directly associated with the negative character of the SO_2 group." Seydel, Krüger-Thiemer, and Wempe (2, 3) obtained the polarizability of the SO_2 group by infrared spectrophotometric measurement of the S–O force constant. They reported that the SO_2 polarizability had no relation to the *in vitro* bacteriostatic activity of the sulfonamides, and the hypothesis of Bell and Roblin is thus cast into doubt. However, the experimental data which led to the Bell and Roblin hypothesis—namely, the correlation of activity with pKa is widely accepted (4, 5). Implications which are derived from the data of Bell and Roblin, and which are considered to be valid are: (*a*) a sulfonamide must possess a pKa which lies within a definite pKa region (6.0–7.5) in order to exhibit maximum activity; (*b*) sulfonamides having a pKa which lies to either side of this region exhibit decreasing activities.

Seydel (6) has recently shown that the *in vitro* bacteriostatic activities for an homologous series of N^1-phenylsulfanilamides are linearly correlated with their respective Hammett σ values. The correlation obtained provided a line of negative slope. In the present report, the authors present other correlations, obtained from the data of Bell and Roblin (1) and supplemented by the data of Seydel, Krüger-Thiemer, and Wempe (2, 3, 6, 7), whose activities correlate linearly with both pKa

and with σ values[1] and for which lines of both positive and negative slopes are obtained. In light of these data, it is pointed out that there is little evidence to support the implications derived from the Bell and Roblin correlation; *i.e.*, that a sulfonamide must have a *definite* pKa (in the range of 6.0–7.5) in order to exhibit maximum activity.

If one classifies the activity-pKa data of Bell and Roblin in terms of homologous series it is noted that: (*a*) in the region where pKa = 6–11, the Bell and Roblin "curve" actually consists of a number of incomplete homologous series, each of which describes a straight line of negative slope; (*b*) in the region where pKa = 2–6, a limited number of homologous compounds can be found (we were able to find no more than three) to which a line of positive slope can be ascribed. In these terms, for the maximum in the Bell and Roblin curve to constitute a true maximum, it would be expected that one set of homologous series would afford lines of positive slope (pKa 2–6), another set of homologous series would afford lines of negative slope (pKa 6–11), and each set would intercept at the accepted maximum.

A search of the literature was made in an attempt to find results which would provide lines of positive slope to substantiate the Bell and Roblin maximum. Only a very limited number of antibacterial activity and pKa data could be found for use in this study. The compiled data (Table I) are presented graphically in Fig. 1; the curved dashed line is that originally reported in the work of Bell and Roblin (1). From the information at hand, only one very incomplete series, the 2-sulfanilamido-(substituted) thiadiazoles, could be said to support the ascending portion (pKa 2–6) of the Bell and Roblin plot.

Two other series appear to cast some doubt on the validity of the Bell and Roblin correlation; the N^1-(substituted) methylsulfanilamide series, which give a line of positive slope and which lies at the