ANTI-BACTERIAL ACTIVITY OF *RAUWOLFIA* ALKALOIDS

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RAUWOLFIA has come to occupy a prominent place in the therapeutics of high blood pressure[1–3] and psychic disorders.[4–7] While the use of this plant in hypertension is only of recent origin, the root had been employed for centuries in Indian medicine for the relief of various central nervous derangements, both psychic and motor, including anxiety, excitement, maniacal behaviour associated with psychosis and epilepsy. Removal of corneal opacities has been observed when the juice from the leaves of the plant were instilled into the eyes.[8] *Rauwolfia* has also been considered as specific for bowel disorders, including diarrhœas, dysentery and cholera. It has also been used as an anti-pyretic.[9]

Since this plant, *R. serpentina*, is mentioned as being very commonly used for bowel disorders, it is rather strange that no mention has been made as to the probable usefulness or otherwise of the drug in these clinical conditions, in spite of the intensive research work going on for the past ten years. Since specific remedies in the form of antibiotics and sulpha drugs are already available for such ailments, attention has probably been diverted towards hypertension and mental conditions for which no specific cure is yet available. However, it is known that many alkaloids possess antimicrobial activity, and have been used for various protozoal infections as specific remedies, *e.g.*, quinine in malaria, emetine in amœbic dysentery, and some of them also possess antibacterial activity.[10]

The alkaloids from the root were extracted with ammoniacal ethylene dichloride, the extract evaporated to dryness *in vacuo*, and the crude total alkaloids thus obtained were extracted with alcohol, filtered and once again dried *in vacuo*. The crude alkaloids have been found to be pharmacologically active in our other studies.[11,12] Reserpine, a pure crystalline alkaloid, was also investigated for its antibacterial properties. An 1% solution of the crude alkaloids in alcohol and a similar solution of reserpine in propylene glycol-alcohol-water mixture (1 : 1 : 2) were initially prepared from which further required dilutions were made. In every case, controls were run with the solvents alone. Results are presented in Tables I and II.

All the strains of organisms used were obtained from the King Institute of Preventive Medicine, Guindy, Madras, except the $H_{37}R_v$ strain of *Mycobacterium tuberculosis* var. *hominis* which was received from the National Collection of Type Cultures, Colindale, England. Youman's medium was used to evaluate the antitubercular activity by surface culture technique.[13] Filter-paper discs, saturated with drugs to be tested and placed on seeded plates of nutrient-agar and blood-agar, were used to determine the inhibitory action on other micro-organisms.

The results may be summarised as follows:

TABLE I

Bacteriostatic action of Rauwolfia *alkaloids* in vitro

Substance	Test Organisms								
	Staph. aureus	*Esch. coli*	*Eberth. typhosum*	*Eberth. paratyph.A*	*Eberth. paratyph.B*	*Shig. sonne*	*Shig. flexneri*	*Shig. shiga*	*Shig. schmitz*
Total alkaloids :									
1 : 10 dilution	22	18	16	12	20	23	10	12	10
1 : 100 dilution	20	13	15	11	17	13	10	10	9
Alcohol (solvent used above for)	–	13	15	11	10	–	–	–	–
Reserpine 1 : 10 dilution	13	–	–	–	–	–	–	–	–
Propylene glycol (solvent for reserpine)	12	–	–	–	–	–	–	–	–

Figures indicate zone of inhibition in mm.; – = No inhibition of growth.

TABLE II

Antitubercular activity of Rauwolfia *alkaloids*

(Youman's media : Surface culture method)

$H_{37}R_v$ strain. Readings at the end of 3 weeks

Concentration 1 in	Total alkaloids	Alcohol (solvent for total alkaloids)	Reserpine	Pr. Glycol mixture (solvent for reserpine)
1,000	–	–	–	–
10,000	–	–	++	++
100,000	+	++	++	++
1,000.000	++	++	++	++
Control	++	++	++	++

– = No growth; + and ++ various grades of growths.

(a) the total alkaloids have activity against all the organisms tested.

(b) *Staphylococcus aurcus* and *Shigella sonne* are more susceptible than the other organisms.

(c) reserpine in 1/10 dilution has no antimicrobial property.

(d) no appreciable difference in the inhibitory concentration is noticed between the alkaloids and the solvents used against *Myco tuberculosis* $H_{37}R_v$. Hence, the alkaloids can be considered to be without anti-tubercular activity.

It is particularly significant that the total alkaloids inhibit the growth of *Staphylococci* and *Shigella sonne*. Many outbreaks of diarrhœas are, of late, being attributed to these two organisms, and hence, the use of *Rauwolfia* decoctions in such conditions may be explained. However, controlled clinical trials are essential to translate the "*in vitro* activity" to therapeutic use.

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USE OF HEAVY WATER IN ORGANIC CHEMISTRY

IN the organic synthesis section, Division of Pure Chemistry, National Research Council, Canada, the following organic compounds labelled with deuterium have been synthesized for use in chemical kinetics, photochemistry and spectroscopy.

(1) Decomposition of the carbide Mg_2C_3 with deuterium oxide gives an excellent yield of propyne-d, $CD_3C \equiv CD$. Several other compounds can be prepared from this material. For instance, chlorination gives 1, 1, 2, 2-tetrachloropropane-d_4, ($CD_3CCl_2CDCl_2$) from which, in turn, 1, 1, 2-trichloropropene-d_2 or *cis*- and *trans*-1, 2-dichloropropane-d_1 can be prepared.

(2) Addition of deuterium bromide to a double or triple bond is another simple method of introducing deuterium into organic compounds. Thus acetylene-d_2 gives a quantitative yield of 1, 2-dibromoethane-d_1. Alternatively, deuterium bromide may be reacted with ordinary acetylene to give 1, 2-dibromoethane-1, 2-d_2. It has been possible to transform both of these compounds into others, e g., ethylene-d_4, ethyl-d- bromide, ethylene-d_4 oxide, etc

(3) Deuteration of organic compounds can also be effected by exchange. Such reactions are catalysed by finely divided metals such as nickel or platinum. For example, benzene is easily deuterated to benzene-d_6 by repeated exchange with deuterium oxide in the presence of platinum black Exchange reactions may also be catalyzed by acids or bases. Trichloroethylene readily exchanges its hydrogen for deuterium when heated with deuterium oxide containing a weak base. An example of an acid-catalyzed reaction is the conversion of malonic acid to malonic-d_2 acid-d_2, namely, $CD_2(CO_2D)_2$.

(4) Sometimes it is more expedient to prepare a compound by reacting a suitable starting material with deuterium oxide and then enriching the product by exchange. For example, about 20 exchanges are required to convert acetone to acetone-d_6. Considerable time is saved by just preparing deuterated acetone (about 90%) from deuteroacetylene and then enriching it by exchange with heavy water.

The greatest difficulties are encountered in the synthesis of compounds labelled with deuterium in a specific position. A discerning choice of starting material must often be made. For instance, when it recently became necessary to prepare butene-1-4, 4, 4-d_3, $CD_3CH_2CH = CH_2$, the problem was solved by reacting the halide $CCl_3.CH_2.CHBr.CH_2Cl$ with zinc and acetic acid-d. In another case, acetaldehyde labelled in the formyl group was prepared by applying Nef reaction to the deuterated nitroethane, $CH_3CD_2NO_2$. The formation of the acetaldehyde-d, disproved a mechanism proposed for the Nef reaction in 1950. These synthetic methods are being extended in several directions (*N R.C. Res. News*, Vol. 8, No. 2).