

CHROM. 11,719

Note

Reduction of pyrrolizidine-N-oxides by the use of a redox polymer

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(Received January 4th, 1979)

In *Boraginaceae*, many mono- and diester alkaloids of the pyrrolizidine type occur as highly water-soluble N-oxides¹. Prior to their qualitative and quantitative analysis, they are usually reduced by the action of zinc and sulphuric acid.

The reduced tertiary bases are easily extractable from a basic aqueous solution with chloroform. Sulphurous acid has also been used as a reducing agent for N-oxides, but might give rise to decomposition². Starodubtseva *et al.*³ used a redox polymer consisting of 2-methyl-5-vinylpyridine and *m*-diisopropenylbenzene, which gave better yields than zinc and acid owing to its milder action in the reduction of platyphylline- and seneciophylline-N-oxide.

Deagen and Deinzer⁴ described a convenient method for the rapid isolation of pyrrolizidine alkaloids in a modification of the earlier method of Mattocks⁵ by the use of ion exchangers. A simple on-column conversion of N-oxides into their corresponding tertiary bases should be of great value in this isolation procedure.

Three possible steps in a column reduction could be incorporated into extraction schemes:

- (1) reduction of the basic eluent from ion-exchange columns;
- (2) reduction of the acid extract from ion-pair extraction for cleaning up of crude alkaloid extracts; and
- (3) direct reduction of N-oxides in methanolic solution in the extraction procedure by means of the method of Deagen and Deinzer⁴.

Because the previous redox polymer³ was unobtainable, we searched for another easily purchasable product. Serdoxit, an adsorbate of indigo disulphonate on a highly porous anion exchanger, proved to be suitable, giving higher yields of reduced alkaloids using method (3) above than the reduction using zinc and acid.

EXPERIMENTAL

Isolation of alkaloids

For isolation of pyrrolizidine alkaloids and their corresponding N-oxides, *Radix consolidae* (*Symphyti radix*) from Ceasar and Loretz (Hamburg, G.F.R.) was used. A 30.0-g amount was extracted with 100 ml of methanol in a Soxhlet extractor for 24 h. The methanol extract was divided into two equal parts, in any experiment one part being reduced by the zinc-acid method and the other by the Serdoxit method.

Reduction of N-oxides

(A) *Reduction by the zinc-acid method.* The methanol was evaporated *in vacuo* and the residue was extracted by shaking with one 50- and two 25-ml volumes of 2 N sulphuric acid and filtered. To the acid extract were added 2.5 g of zinc dust, followed by stirring for 3 h at room temperature.

(B) *Reduction with Serdoxid (Serva, Heidelberg, G.F.R.).* (1) An acid extract prepared as described under (A) was adjusted to pH 9.5 with ammonia solution. This extract was passed through a column (25 × 1.8 cm I.D.) of reduced Serdoxid at a flow-rate of 1 ml/min and eluted with demineralized water.

(2) An acid extract prepared as described under (A) was passed through a Serdoxid column and eluted with demineralized water.

(3) The methanolic extract obtained after Soxhlet extraction was passed through a Serdoxid column and eluted with methanol.

The eluate from procedure (B3) was treated as described under (A), except for the reduction step. The final products from procedures (A), (B2) and (B3) were adjusted to pH 9.5 with ammonia solution. The separate basic solutions were extracted with chloroform (one 50- and two 25-ml volumes), then the chloroform phase was dried over sodium sulphate. The filtered, sodium sulphate-free chloroform phase was evaporated *in vacuo* and 2.0 ml of chloroform were added.

Regeneration of Serdoxid columns

The regeneration of exhausted (black) Serdoxid columns was performed by elution with three times the column volume of a solution of 5% (w/w) sodium dithionite in demineralized water. Upon regeneration the column turned light brown. Prior to use, the column was washed with demineralized water and, in the reduction of methanolic extracts, subsequently with methanol.

Thin-layer chromatography (TLC) and scanning of reduced alkaloids

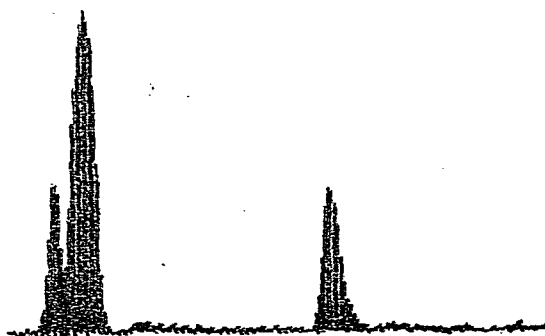
A 1- or 2- μ l volume of the final extract of reduced pyrrolizidine alkaloids was spotted on a silica gel G 1500 TLC plate (20 × 20 cm, Schleicher & Schüll, Dassel, G.F.R.) by means of a Camag Nanomat spotter. The eluent for plate development according to Pedersen¹ (chloroform-methanol-25% ammonia solution, 85:14:1) was used. After a run of approximately 10 cm the plate was air dried and dipped for 20 sec in a mixture of Dragendorff reagent (Munier, not diluted)⁶, acetone and acetic acid (60:12:1).

After drying in a stream of air, the plate was scanned by means of a Shimadzu CS-910 TLC scanner. The optimal wavelengths of the sample and reference beam had been determined to be 510 and 660 nm, respectively. The zig-zag scan speed was 10 mm/min. Measurements were performed in the dual-beam reflection absorption mode.

RESULTS AND DISCUSSION

From Fig. 1, which compares the zinc-acid and basic Serdoxid reduction, it can be clearly seen that the latter gives much lower yields of reduced alkaloids.

The reduction of N-oxides in acidic solution gave appreciable yields of symphytine and echimidine but variable results for echinatine. The reduction of a metha-



9 0



A B

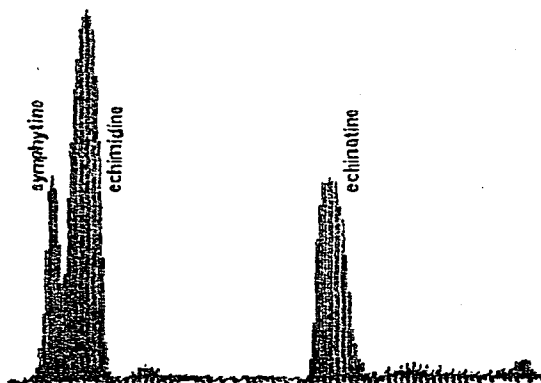


Fig. 1. Thin-layer chromatogram of total pyrrolizidine alkaloid extract from *Radix consolidae*. A, after zinc-acid reduction of N-oxides; B, after reduction according to procedure (B1). Amount spotted: 2 μ l.

Fig. 2. Zig-zag scans of a chromatogram as in Fig. 1. Upper scan, after zinc-acid reduction of N-oxides; lower scan, direct on-column reduction of N-oxides in a methanolic extract. Amount spotted: 2 μ l.

nolic extract in all experiments gave higher yields of tertiary bases than the zinc-acid method, as shown in Fig. 2 by the ratio of the respective TLC peak areas.

The on-column reduction by means of a readily available redox polymer, which can be regenerated and which is mild in the reduction of N-oxides, makes it readily applicable to the isolation procedure of Deagen and Deinzer⁴.

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