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# Phytochemical investigations of some species of *Colchicum*

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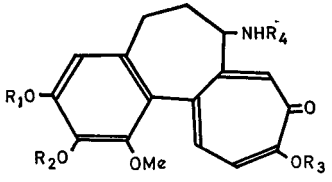
A colorimetric assay for colchicine and its congeners has been devised. It is based on the yellow colour produced by the treatment of these alkaloids with mineral acid and is useful over the range 0·0005–0·0025% w/v. Together with the method of Pesez (1957) it has been used to assay extracts of species of *Colchicum* and the individual alkaloids were identified by paper chromatography. It was concluded that *C. tunicatum*, a native of Israel, contained sufficient colchicine to make it a useful source of this alkaloid.

THE presence of colchicine and many congeners in numerous species of *Colchicum* and other liliaceous plants was reported by Šantavý (1957). The alkaloids which contain the tropolonic ring C are either of the colchicine-type (I; $R_3$ = Me) or the colchiceine-type (I; $R_3$ = H). Each class in turn may be basic, neutral or phenolic.

Species of *Colchicum* indigenous to Israel have been reported to contain large amounts of colchicine (Boyko, 1954) and several have been investigated previously. Šantavý, Černoch, Lang, Malinský & Zajíčková (1951) isolated and assayed colchicine (I; neutral) and demecolcine (I; basic) from the corms of *C. cilicum* Hayek [= *C. steveni* Kunth (Stefanoff, 1926)] whilst Kaul, Moza, Šantavý & Vrublovský (1964), using paper chromatography, reported the presence of tropolonic alkaloids in *C.*




I

| Alkaloid | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Colchicine .. .. | Me | Me | Me | COMe |
| Colchiceine .. .. | Me | Me | H | COMe |
| Demecolcine .. .. | Me | Me | Me | Me |
| Substance C .. .. | H | Me | Me | COMe |
| Substance $E_1$ .. .. | Me | H | Me | COMe |
| Substance B .. .. | Me | Me | Me | CHO |
| Substance S .. .. | Me | H | Me | Me |

*ritchii* R. Br. *C. hierosolymitanum* Feinbr. has also been investigated and the amount of colchicine determined (Weizmann, 1952; Šantavý, Hoščálková, Podivínský & Potěšilová, 1954; Šantavý, Zajíček & Nemečková, 1957).

The aim of our investigation was threefold: (1) to evolve a rapid, colorimetric assay for tropolonic alkaloids of both the colchicine- and

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colchiceine-types; (2) to determine the amount of alkaloids in some species of *Colchicum* from Israel; (3) to identify the individual alkaloids of both classes by paper chromatography. As Israeli samples were small, samples of more readily-available species of *Colchicum* were included to ascertain that the assay procedures used were of value with only one small sample of the drug.

### MATERIALS

Specimens of *C. ritchii* R. Br., *C. steveni* Kunth and *C. tunicatum* Feinbr. were received from Prof. M. Evenari and Dr. N. Feinbrun, the Hebrew University, Jerusalem, Dr. J. Galil, Tel-Aviv University, and the Tropical Products Institute, London. These were raised in a heated greenhouse. *C. ritchii* and *C. tunicatum* did not flower and leafy plants were used. *C. steveni* produced healthy, flowering plants and these were used whole. Corms of *C. autumnale minor* and *C.* hybrid Disraeli, from a bulb-grower, were raised in a garden in Bradford; plants in flower were used. *C. autumnale* L. was a commercial sample of colchicum corm. It contained much corm scale and other extraneous material. When assayed by the method of the *British Pharmacopoeia* (1963) it yielded only 0·20% of the alkaloids of colchicum corm (official limits: not less than 0·25%). *C. luteum* Bak. was donated by Prof. G. E. Trease, the University, Nottingham, and consisted of corms, devoid of scales, dried at a temperature sufficient to gelatinise the starch.

## Phytochemical investigations

### EXTRACTION PROCEDURES

Plants were harvested, washed and dried (30°). The material was then divided into flowering tops and underground organs or left whole, and then reduced to a coarse powder.

The powder (5 g) was extracted (3–4 hr) in a Soxhlet with ethanol (90%) (150 ml). Ethanol was evaporated under reduced pressure (60°) and the residue dissolved in distilled water and the solution filtered.

The solution was shaken with ether (6 × 20 ml), made just acid with dilute hydrochloric acid and shaken with chloroform (2 × 20 ml), made alkaline with concentrated ammonia solution and extracted with chloroform (6 × 20 ml). At this stage, all tropolonic alkaloids had been removed from the aqueous phase which did not give a lemon-yellow colour with concentrated hydrochloric acid.

Ether fractions were bulked, washed with distilled water (20 ml) and hydrochloric acid (5% v/v) (20 ml); the ether solution was then discarded.

Chloroform fractions were bulked, washed with distilled water (20 ml), hydrochloric acid (5% v/v) (20 ml), distilled water (20 ml) and aqueous sodium carbonate (10% w/v) (20 ml). The residual chloroform solution containing neutral and phenolic alkaloids was evaporated to dryness (100°), the residue dissolved in ethanol (2 ml) and again evaporated to dryness.

The acid washings from the ether and chloroform solutions containing basic alkaloids were combined, neutralised with solid sodium bicarbonate

and extracted with chloroform (2 × 20 ml), which removed all tropolonic alkaloids from the aqueous layer. The chloroform solution was evaporated to dryness (100°), the residue dissolved in ethanol (2 ml) and again evaporated to dryness.

The residues containing (i) basic and (ii) neutral and phenolic alkaloids were dissolved in ethanol (2 ml) for paper chromatography and in distilled water and made to 50 ml for colorimetric assay.

### PAPER CHROMATOGRAPHY OF EXTRACTS

Whatman No. 1 paper and ascending technique were used. Papers were examined in ultraviolet light (360 mμ) and then treated with the vapour from concentrated hydrochloric acid or fuming nitric acid which coloured the spots of tropolonic alkaloids lemon-yellow. There has been no reference to the use of this spray reagent but Giebelmann (1964) used a spray of Millon's Reagent for colchicine and the yellow colour of the spots was presumably due to the concentrated nitric acid present.

Authenticated specimens of colchicine,* demecolcine† and colchiceine were used as reference substances. Solvent systems used, spot colours and Rf values are listed below.

A. Distilled water (method of Salo, 1960): Rf values and spot colours found for colchicine, demecolcine, colchiceine were respectively 0·71–0·82 (yellow in ultraviolet light and after spray), 0·37–0·49 (green-blue in ultraviolet light, yellow after spray), 0·59–0·64 (yellow in ultraviolet light and after spray). Salo (1960) quoted Rf values for colchicine of 0·7–0·8 and for demecolcine of 0·4–0·5.

B. n-Butanol-acetic acid-water (4 : 1 : 5) (method of Delong, Havriliková & Šantavý, 1955): Rf values and spot colours found; colchicine, colchiceine (yellow in ultraviolet light and after spray) travelled with solvent front (diffuse band); demecolcine (greenish-yellow in ultraviolet light, yellow after spray) was 0·70–0·78. Delong & others (1955) quoted 0·79 for demecolcine.

### COLORIMETRIC ASSAYS OF EXTRACTS

*Method of Pesez* (1957). Into each of 6 tubes was placed 5, 4, 3, 2, 1, 0 ml of aqueous alkaloid (0·001% w/v) and each was diluted to 5 ml with water. To each tube was added aqueous isoniazid (10% w/v) (2 ml) and aqueous sodium carbonate (10% w/v) (1 ml). The tubes were heated (10 min) to 100°. The absorbance of the resulting deep orange-yellow solution was measured at 450 mμ, using the tube without alkaloid to set zero absorbance, in a Bausch & Lomb Spectronic 20 colorimeter. A calibration graph was drawn for colchicine and demecolcine. Extracts were treated similarly, after suitable dilution with water, and readings compared with the standard curve. The method assays alkaloids of the colchicine-type.

*Proposed method.* The lemon-yellow colour produced by treating colchicine with mineral acid (Hübler, 1865) arises from the presence of

* B.D.H. Laboratory Reagent.
† Colcemid CIBA Laboratories.

the tropolone ring C (Pauson, 1955). With colchicine and demecolcine we found that the absorbing species has a λmax 385 mμ and when the colour is produced under standard conditions the absorbance at this wavelength is directly proportional to the amount of alkaloid present. The linear relationship holds over the range 0·0005–0·0025% w/v alkaloid.

Into each of 6 tubes was placed 5, 4, 3, 2, 1, 0 ml aqueous alkaloid (0·005% w/v) and each was diluted to 5 ml with water. To each tube was added hydrochloric acid (s.g. 1·18) (5 ml) and the absorbance of the lemon-yellow colour, which developed immediately, was measured, using the tube without alkaloid to set the zero absorbance. A calibration curve was drawn for colchicine and demecolcine. Extracts were treated similarly, after suitable dilution with water, and the readings compared with those of the standard curve. This estimates those alkaloids of the Liliaceae which contain a tropolone ring (Šantavý, 1957) whether of the colchicine, or colchiceine type. Thus the difference between the assay figures given by the two methods may be taken as the amount of colchicine-type alkaloids present.

## Results

Assay figures and alkaloids identified by paper chromatography are presented in Table 1.

By the paper chromatographic method of Salo (1960) a spot of Rf 0·009–0·34, fluorescing blue in ultraviolet light was observed. This is a very wide range of values but, as comparison with the chromatograms obtained by the method of Delong & others (1955) showed only one blue, fluorescent spot of Rf 0·82–0·84, it was considered that only one substance was present and that this was Substance C (I; $R_1$ = H, $R_2$ = $R_3$ = Me, $R_4$ = COMe) (phenolic) reported by Delong & others (1955) to have an Rf 0·84 and to fluoresce bright blue on paper chromatograms. Water is a poor solvent system for this substance.

## Discussion

We have calculated from the results of Šantavý & others (1954) that, in the corms of *C. autumnale*, of all neutral and phenolic alkaloids containing the tropolone ring, 90% is colchicine, whilst 98% of the basic alkaloids is demecolcine. Other tropolonic alkaloids have been detected in species of *Colchicum*, but for most species substances isolated or detected have been colchicine, colchiceine, demecolcine and Substance C and/or $E_1$ (I; $R_1$ = $R_3$ = Me, $R_2$ = H, $R_4$ = COMe) (phenolic) and more rarely Substance B (I; $R_1$ = $R_2$ = $R_3$ = Me, $R_4$ = CHO) (neutral) and Substance S (I; $R_1$ = $R_3$ = $R_4$ = Me, $R_2$ = H) (basic/phenolic but found in basic fractions). The average amount of C and/or $E_1$ is 0·0007–0·0033% and so an assay for tropolonic alkaloids is virtually one for colchicine and demecolcine. This was verified by paper chromatography where the only tropolonic compounds detected were colchicine, colchiceine, demecolcine and Substance C. The colour with the spray was only prominent for colchicine and demecolcine, showing that these

TABLE 1. RESULTS OF COLORIMETRIC ASSAYS AND PAPER CHROMATOGRAPHY OF EXTRACTS OF SPECIES OF *Colchicum*.*

| Plant and state | Colorimetric assays: Neutral/phenolic alkaloids: Tropolonic (Present method) | | Colorimetric assays: Neutral/phenolic alkaloids: Colchicine type (Pesez (1957)) | Colorimetric assays: Basic alkaloids: Troplonic (Present method) | Colorimetric assays: Basic alkaloids: Colchicine type (Pesez (1957)) | Alkaloids identified by paper chromatography |
|---|---|---|---|---|---|---|
| *C. autumnale L* (Colchicum corm) | | | | | | Colchicine AB** |
| Mean | 0·101 | (12)† | 0·097 | 0·098 | 0·095 | Colchiceine B |
| Standard deviation | 0·006 | | 0·009 | 0·006 | 0·005 | Substance C AB |
| Mean colchiceine-type alkaloids | 0·004 | | | 0·003 | | Demecolcine AB<br>Colouring matter A |
| *C. autumnale minor* corms | | | | | | |
| Mean | 0·080 | (8) | 0·079 | 0·106 | 0·101 | Colchicine AB |
| Standard deviation | 0·009 | | 0·005 | 0·005 | 0·009 | Colchiceine B |
| Mean colchiceine-type alkaloids | 0·001 | | | 0·005 | | Substance C AB<br>Demecolcine AB |
| Flowering tops | | | | | | |
| Mean | 0·304 | (8) | 0·282 | 0·122 | 0·103 | Colchicine AB |
| Standard deviation | 0·043 | | 0·012 | 0·003 | 0·014 | Colchiceine AB |
| Mean colchiceine-type alkaloids | 0·022 | | | 0·038 | | Substance C AB<br>Demecolcine AB<br>Colouring matter A |
| Whole plant in flower | | | | | | |
| Mean | 0·112 | (8) | 0·091 | 0·092 | 0·087 | Colchicine AB |
| Standard deviation | 0·003 | | 0·001 | 0·003 | 0·003 | Colchiceine AB |
| Mean colchiceine-type alkaloids | 0·021 | | | 0·005 | | Substance C AB<br>Demecolcine AB<br>Colouring matter AB |
| *C.* hybrid Disraeli corms | | | | | | |
| Mean | 0·032 | (6) | 0·028 | 0·034 | 0·032 | Colchicine AB |
| Standard deviation | 0·002 | | 0·001 | 0·003 | 0·002 | Colchiceine AB |
| Mean colchiceine-type alkaloids | 0·004 | | | 0·002 | | Substance C AB<br>Demecolcine AB |
| Flowering tops | | | | | | |
| Mean | 0·347 | (2) | 0·323 | 0·095 | 0·093 | Colchicine AB |
| Standard deviation | 0·012 | | 0·004 | 0·006 | 0·006 | Colchiceine AB |
| Mean colchiceine-type alkaloids | 0·024 | | | 0·002 | | Substance C AB<br>Demecolcine AB<br>Colouring matter A |
| Whole plant in flower | | | | | | |
| Mean | 0·057 | (6) | 0·040 | 0·039 | 0·033 | Colchicine AB |
| Standard deviation | 0·002 | | 0·002 | 0·001 | 0·002 | Colchiceine AB |
| Mean colchiceine-type alkaloids | 0·017 | | | 0·006 | | Substance C AB<br>Demecolcine AB<br>Colouring matter A |
| *C. luteum* Bak. | | | | | | |
| Mean | 0·049 | (6) | 0·047 | cannot be | 0·004 | Colchicine AB |
| Standard deviation | 0·002 | | 0·003 | assayed | 0·001 | Colchiceine AB |
| Mean colchiceine-type alkaloids | 0·002 | | | by this method | | Substance C B<br>Colouring matter A<br>‡3 unidentified substances |
| *C. ritchii* R. Br. whole, leafy plant | 0·055 | (1) | 0·050 | 0·060 | 0·056 | Colchicine AB |
| Colchiceine-type alkaloids | 0·005 | | | 0·004 | | Colchiceine AB<br>Substance C AB<br>Demecolcine AB |
| *C. steveni* Kunth whole, flowering plant | 0·074 | (1) | 0·068 | 0·033 | 0·030 | Colchicine AB |
| Colchiceine-type alkaloids | 0·006 | | | 0·003 | | Colchiceine AB<br>Substance C AB<br>Demecolcine AB |
| *C. tunicatum* Feinbr. whole, leafy plant | 0·160 | (1) | 0·155 | 0·56 | 0·050 | Colchicine AB |
| Colchiceine-type alkaloids | 0·005 | | | 0·006 | | Colchiceine AB<br>Substance C AB<br>Demecolcine B<br>Colour matter A |

(See next page for footnotes)

are the only tropolonic compounds present in large quantities, except in flowering tops where large amounts of colchiceine were detected.

There was only sufficient for one determination of each Israeli sample, therefore this work can constitute only a preliminary investigation. However, both assay methods used gave reasonably consistent results with other species of *Colchicum*. Those for *C. autumnale*, *C. autumnale minor* corms and *C. luteum* show good correlation with published results (Šantavý & others, 1951, 1954; Delong & others, 1955; Šantavý, 1957; Yusupov & Sadykov, 1962). There was an exception with the flowering tops of *C. autumnale minor* which show a large standard deviation when assayed by our method. The results were much lower than those quoted for the flowers of *C. autumnale* (Šantavý & Mačák, 1954) and in the basic fraction we found that the major alkaloid was demecolcine whereas Šantavý & Mačák (1954) quoted Substance S.

*C.* hybrid Disraeli. Šantavý & others (1951) reported that the colchicine contents of the hybrids Lilac Wonder, The Giant and Violet Queen were 0·47, 0·095, 0·113% and the contents of demecolcine were 0·014, 0·014 and 0·067% respectively. For the Disraeli hybrid, we found the content of neutral and phenolic alkaloids of the colchicine-type to agree most closely with that found for the hybrid The Giant but none of the hybrids had a comparable amount of demecolcine. It is therefore possible that both The Giant and Disraeli hybrids have one parent in common which carries the dominant gene for the quantity of colchicine, whereas the parent carrying the dominant gene for the amount of basic alkaloids is different. The low amount of alkaloids in the corm is desirable in a garden plant but this is to some extent off-set by the large amount of alkaloids in the flowers which may prove attractive to children.

*C. ritchii.* Fahmy (1963) reported that the corms of this species contained only traces of colchicine. The total amount of neutral and phenolic alkaloids, which we have identified as colchicine, colchiceine and Substance C was 0·055% of which 0·050% was colchicine and Substance C. As Substance C was not present in sufficient amounts to react with the spray reagent after chromatography, colchicine must be present in much the greater amount. Thus, this species contains more than traces of colchicine, but not sufficient amounts to make it a valuable source of the alkaloid. Comparatively large amounts of demecolcine were present and the species would be a reasonably good source of this alkaloid.

*C. steveni.* Šantavý & others (1951) isolated 0·044% colchicine and 0·039% demecolcine from the corms of *C. cilicum* Hayek [= *C. steveni* Kunth (Stefanoff, 1926)]. Our results, from the whole plants, show a

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*Footnotes to Table 1.*

* All neutral and phenolic alkaloids are expressed as percentage of colchicine and basic alkaloids as percentage of demecolcine. All results are calculated with reference to oven-dried material. The assays for each group were performed on one sample.

** Alkaloids shown present by paper chromatography with solvent system A, distilled water: B, n-butanol-acetic acid-water (4 : 1 : 5).

† number of readings.

‡ Spots of Rf (A) 0·00, 0·63, 0·77; (B) 0·21, 0·75, travelling with solvent front, believed to be some of the non-tropolonic bases reported by Sadykov & Yusupov (1965).

higher concentration of colchicine, 0·068%, but a comparable amount of demecolcine, 0·030%. The plants used by Šantavý & others (1951) were obtained from a bulb merchant and it is probable that they had been under cultivation for several years; the corms we used were cultivated for one year only. Salo (1963) observed that *C. laetum* Stev., when cultivated, yielded less colchicine than in the wild state and it is possible that this is a feature of *C. steveni* also. It is considered doubtful that the presence of the flowers, even if these contain much more colchicine than the flowers of other species examined, would raise the alkaloidal content to 50% more than previously reported results.

*C. tunicatum.* No work has been published on the alkaloidal content of this species. We found the whole plant to contain large amounts (0·155%) of neutral and phenolic alkaloids of the colchicine type. This is comparable to the colchicine content of *C. autumnale*, 0·15%, obtained by Šantavý (1950). Besides colchicine, *C. tunicatum* contains Substance C, but as this latter gave no colour with the spray reagent on paper chromatograms, it was concluded that it was only present in trace amounts. The results suggest that *C. tunicatum* contains quantities of colchicine sufficient to be a good source of this alkaloid. It also contains comparatively large amounts of demecolcine.

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