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THE STRUCTURE AND SYNTHESIS OF SPECIOSINE

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Abstract—The structure of speciosine (**1**) was deduced by spectroscopic methods and confirmed by synthesis from demecolcine (**2**) and 2-bromomethylphenyl acetate (**6**).

DURING an extensive study of the biosynthesis[1] of demecolcine (**2**), colchicine (**3**) and related alkaloids found in species belonging to the family *Lilliaceae*, it was decided to investigate the structures of several minor alkaloids from this source, in the hope that these would give support for our biosynthetic proposals. Speciosine is a minor alkaloid found[2] to co-occur with demecolcine (**2**) and colchicine (**3**) in *Colchicum speciosum*. Although unable to assign a structure to speciosine, Kiselev determined that it was a weak base, $C_{28}H_{31}NO_6$, incorporating a OH and four OMe groups.

In the course of our isolation work, we were able to confirm that the amount of speciosine in C. *speciosum* varies seasonally. The small quantities of the alkaloid available (~60 mg.) precluded the use of degrative procedures and only permitted use of spectroscopic methods, coupled with biosynthetic argument, in structure elucidation.

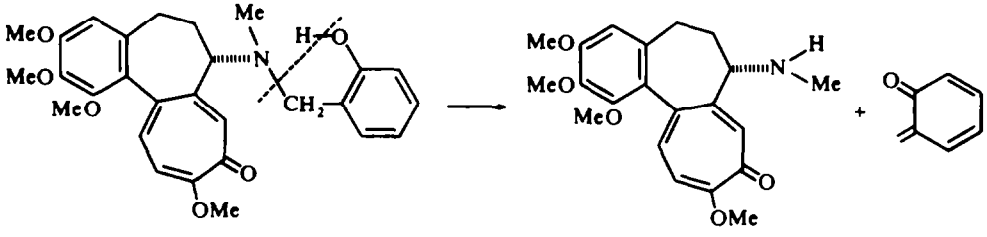
The UV absorption maximum at 350 nm in speciosine suggested the presence of the o-methyl tropolone system present in demecolcine (**2**) and colchicine (**3**) and found in several related alkaloids of the *Lilliacae*. Addition of alkali caused a shoulder at 275 nm to shift to 293 nm, indicating the presence of phenolic group. These structural features were supported by the IR spectrum, which exhibited a characteristic group of absorptions at 1610, 1595, 1575 $cm.^{-1}$ also found in demecolcine (**2**) and attributable to the o-methyl aryl tropolone system. The IR also suggested that the phenolic OH was intramolecularly hydrogen-bonded.

Confirmation of the molecular formula, $C_{28}H_{31}NO_6$, was given by the mass spectrum of speciosine, which gave a parent ion at *m/e* 477. More important, however, was the information gleaned from the fragmentation pattern. Again the similarity with demicolcine[3] (**2**) was striking, in that the base peak was found to be *m/e* 207 (100). The metastable ion corresponding to the fragmentation 371 — 207 was found at *m/e* 115·5. Other important fragments were *m/e* 462 (**3**), 371 (60), 107 (33), 106 (33). It thus appears that speciosine mainly fragments by loss of $—C_7H_7O$ or $—C_7H_6O$, which involves a hydrogen transfer process leading to the observed ion *m/e* 371. From the intensity and ease of formation, it was thought that the C_7H_7O moiety was a substituted benzyl group.

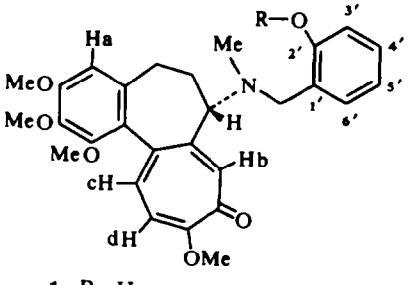
From considerations of UV, IR and mass spectra a partial structure (**4**) emerged, leaving only the nature of the C_7H_7O residue to be determined. The NMR spectrum of speciosine clearly indicated N-CH_3 (7·82 τ), three O—CH_3 (6·10, 6·14, 6·50 τ)

and eight aromatic H (2·32–3·4 τ). All the aromatic protons of the demecolcine (**2**) moiety could easily be identified Ha, 3·49; Hb, 2·32; Hc, 3·29 (d, J = 11 cps.); Hd 2·80 τ (dl J = 11 cps.) leaving a three H multiplet at ~3·3 τ and, most significantly, a single H as a quartet at 2·96 τ (J = 7, 1·5 cps). This 3:1 ratio of aryl protons in the C_7H_7O portion of speciosine can best be fitted by placing the OH function at 2', as represented by structure (**1**) for speciosine. Thus the low field proton quartet at 2·96 can be attributed to the 3'-H, being *ortho* to the phenolic group and subject to both *ortho* (7 cps.) and *meta* 1·5 cps) coupling to be the 4'-H and 5'-H.

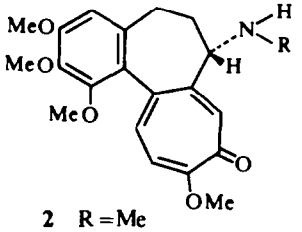
The postulated structure for speciosine (**1**) would explain the hydrogen bonded OH group and also the mass spectral fragmentation 477 → 371 with loss of *m/e* 106 rather than 107, which would be expected from complete fission of the *ortho*-hydroxybenzyl fragment.



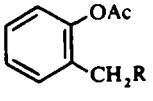
It was decided to verify the postulated structure for speciosine (**1**) by synthesis, utilising demecolcine (**2**) as starting material. This was alkylated, in the presence of K_2CO_3, with 2-bromomethylphenyl acetate (**6**),[4] which had been prepared by the action of N-bromosuccinimide on *ortho*-cresyl acetate (**5**). The acetoxy derivative (**7**) was not amenable to rigorous purification due to facile cleavage of the ester



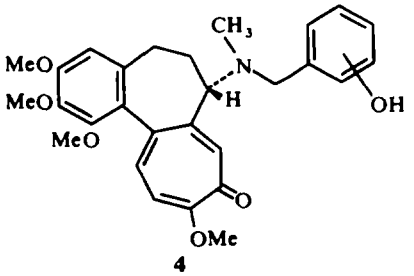
1 R=H
7 R=Ac



2 R=Me
3 R=Ac



5 R=H
6 R=Br



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function. Complete hydrolysis was effected with dilute NaOH aq to afford structure (1), which proved to be identical in all respects with natural speciosine isolated from *C. speciosum.*

From a biosynthetic standpoint, speciosine (1) is of interest because of the N-benzyl moiety with its unusual position of oxygenation. The results of tracer experiments, designed to investigate the origin of this interesting structural feature, will be reported later.

EXPERIMENTAL

All m.p's and b.p's are uncorrected. UV spectra were measured in EtOH on a Unicam SP 800 spectrometer. IR spectra were determined using a Unicam SP 200 spectrometer. NMR spectra were taken on Varian A56–60A and HA 100 spectrometers, (TMS). Optical rotation measurements are for $CHCl_3$ solution using a Bendix–Ericsson ETL-NPL automatic polarimeter type 143A. Petroleum ether refers to the fraction bp 60–80°. Alumina used was Woelm Grade I (Neutral) deactivated with the required amount of water.

Extraction of C. speciosum. Twenty flowering plants (850 g) were extracted with MeOH (8 l) and the solvent removed *in vacuo.* The residue was partitioned between water (250 ml) and petroleum ether (4 × 200 ml). The aqueous layer was then extracted with $CHCl_3$ (1 l), which was dried (Na_2SO_4) and evaporated to give a gum (3·63 g). This was adsorbed on a partition column of celite (200 g): H_2O (100 g, previously saturated with EtOAc/petroleum ether 1:1). Elution with EtOAc/petroleum ether, 1:1 (previously saturated with H_2O) gave a non-polar fraction (380 mg) followed by demecolcine (1·4 g). Elution with EtOAc (previously saturated with H_2O) yielded colchicine (1·0 g).

The non-polar fraction (380 mg) was chromatographed over alumina (grade IV, 9 g). Elution with $CHCl_3$ gave a gum (146 mg), which was re-chromatographed on alumina (grade IV, 9 g). Careful elution with 40% $CHCl_3$ in C_6H_6 afforded material (70 mg), having the same R_f as authentic speciosine (kindly supplied by Dr. F. Santavy) in the tlc system C_6H_6/EtOAc/diethylamine, 7:2:1. This was crystallised from EtOAc/C_6H_6 to give pure speciosine (40 mg) m.p. 211–214°C; $[\alpha]^{2}_{D}{}^{0}$ − 22°; UV λ_{max} 350 nm (log ε 3·74); IR ($CHCl_3$) ν_{max} 3400 (broad), 1610, 1595, 1575 cm.$^{-1}$; NMR ($CDCl_3$) τ 3·49 (Ha), 2·32 (Hb), 3·29 (Hc, d J = 11 cps), 2·80 (Hd, d J = 11 cps), 3·3 (m, 3H), 2·96 (H, q, 7, 1·5 cps), 6·10 (s, 3H), 6·14 (s, 3H), 6·50 (s, 3H), 7·82 (s, 3H). Mass spectrum showed the following important fragments at m/e 477, 462, 371, 207, 107, 106.

2-*Bromomethylphenyl acetate* (6). *Ortho*-cresol (30 g) in acetic anhyd/pyridine (80 ml, 1:1) was allowed to stand for 24 hr. at room temperature. Removal of solvent followed by distillation afforded the ester (5) (30 g) b.p. 105°/20 mm; NMR ($CDCl_3$) τ 3·0 (m, 4H) 7·80 (s, 3H), 7·88 (s, 3H); IR (Film) ν_{max} 1760 cm.$^{-1}$

Ortho-cresyl acetate (10 g) in CCl_4 (30 ml) was refluxed for 1 hr. with N-bromosuccinimide (11·9 g) and a trace of dibenzolyperoxide. After 1 hr. the mixture was cooled, filtered, and the solvent removed *in vacuo* to give an oil, which was distilled to give 2-bromomethylphenyl acetate (6), b.p. 82°/0·02 mm.[4] NMR ($CDCl_3$) τ 2·9 (m, 4H), 5·67 (s, 2H), 7·78 (s, 3H); IR (Film) ν_{max} 1760 cm.$^{-1}$.

Speciosine (1). A solution of demecolcine (200 mg) and 2-bromomethylphenyl acetate (130 mg) in MeCN (7 ml) was stirred with anhydrous K_2CO_3 (200 mg) for 16 hr at room temperature. The reaction mixture was filtered and the solvent removed *in vacuo* to give a gum, which was treated with EtOAc/Et_2O. Again the mixture was filtered, followed by removal of the solvent *in vacuo.* Chromatography of the residue over alumina (grade IV, 5 g) gave the acetoxy derivative (7) (100 mg) on elution with 20% $CHCl_3$ in C_6H_6. This was homogeneous by tlc on silica using C_6H_6/EtOAc/diethylamine 7:2:1; IR ($CHCl_3$ ν_{max} 1760, 1610, 1595, 1575 cm.$^{-1}$. Further elution with 40% $CHCl_3$ in C_6H_6 afforded partially hydrolysed material (150 mg).

The total material (250 mg) was dissolved in MeOH (3 ml) containing 2N NaOH (0·3 ml). After 1 hr at room temperature, the solvent was removed *in vacuo* and H_2O added. The solution was acidified with AcOH extracted with CH_2Cl_2 and the organic layer washed with H_2O then dried (NA_2SO_4). Removal of the solvent gave a solid (230 mg), which crystallised from acetone to give (1) (180 mg; 72%) as yellow crystals m.p. 211-214°C; $[\alpha]_{D}^{20}$ − 24° (C. 1% $CHCl_3$). The synthetic material was identical to natural speciosine in all respects.

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