

THE DISTRIBUTION OF MESEMBRINE ALKALOIDS IN SELECTED TAXA OF THE MESEMBRYANTHEMACEAE AND THEIR MODIFICATION IN THE *SCELETIUM* DERIVED 'KOUGOED'

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ABSTRACT

Twenty species from nine genera of the Mesembryanthemaceae (*Aptenia*, *Bergeranthus*, *Delosperma*, *Drosanthemum*, *Glottiphyllum*, *Lampranthus*, *Oscularia*, *Ruschia*, and *Sceletium*) as well as the reportedly psychoactive preparation 'kougoed', prepared from 'fermenting' *Sceletium tortuosum*, were screened for the presence of the mesembrine alkaloids. Using gas chromatography (GC) with a nitrogen-phosphorous detector (NPD) three putative alkaloids were detected in *Sceletium tortuosum* whose mass spectra corresponded to those of 4'-O-demethylmesembrenol, mesembrine and mesembrenone. All the Mesembryanthemaceae plants investigated were shown to have Dragendorff-positive compounds on thin layer chromatograms (TLC); those containing mesembrine alkaloids, as shown by later GC MS analysis, exhibited similar Rf values to the *Sceletium* alkaloids. However, using the technique employed in this study which encompassed the use of column and gas chromatography, the only genus containing mesembrine alkaloids to any significant extent was *Aptenia*. Alkaloid levels were found to be extremely low in all other taxa investigated. When a 'modern' technique for the prepara-

tion of a fermented *Sceletium* product, 'kougoed', was carried out it was found that levels, as well as the ratios, of the three alkaloids changed markedly. Substantial increases in total alkaloid levels were observed when the *Sceletium* material was crushed and bruised prior to drying for alkaloid extraction whereas no such changes occurred when intact plants were oven dried at 80°C prior to alkaloid extraction. It is speculated that of the many potentially usable Mesembryanthemaceae plants available to the indigenous peoples, *Sceletium* was selected because it is the only genus with alkaloid levels high enough to elicit a psychoactive response. The traditional preparation technique also appears to have evolved as a method of producing a dry, stable, and relatively palatable preparation of increased pharmacological activity.

INTRODUCTION

Humankind has used drugs containing alkaloids in potions, medicines, teas, poultices and poisons for over 4000 years. The use of 'kougoed' amongst the KhoiSan as a psychoactive plant concoction to ward off thirst and use in trade is by no means unique; parallels can be drawn with the use and trade of coca leaves (*Erythroxylum coca* Lam.) amongst the Andeans of South America, who for centuries chewed coca leaves to raise their spirits and ward off hunger and fatigue, as well as trading the item for corn and meat (Allen, 1981).

The psychoactive properties of 'kougoed', a concoction prepared from *Sceletium* N.E.Br. species by the KhoiSan of Southern Africa using a fermentation process, has been recorded in the literature for over 300 years and is the subject of a recent review (Smith et al., 1996). Early accounts described how the preparation of

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Species: *Sceletium tortuosum*; *Aptenia cordifolia*; *Bergeranthus scapiger*; *Ruschia lineolata*; *Lampranthus deltoides*; *Glottiphyllum longum*; *Drosanthemum hispidum*; *D. bicolor*; *Delosperma minimum*; *D. pruinosum*; *D. pottsii*; *D. cooperi*; *D. rogersii*; *D. lebombense*; *D. obtusum*; *Lampranthus aureus*; *L. roseus*; *L. spectabilis*; *L. blandus*; *L. coccineus*

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'kougoed' follows a fermentation process in which whole plant material was crushed between two stones and then placed in sealed containers for several days. Although in the past a skin or canvas bag was used as the fermentation vessel these have been replaced by plastic bags, as recent field studies in Namaqualand have indicated:

"Leave the bag of crushed 'kougoed' in the sun to get warm; it's not necessary to put it (the bag) in the shade, it gets shade at night, and the sun doesn't harm it. The plant is left to sweat. After 2 to 3 days the bag is opened, the 'kougoed' is mixed around, and then the bag is tightly closed again. On the eighth day after the crushing, the bag is opened and the 'kougoed' is spread out to dry in the sun, as when you dry raisins. You leave it out until it is dry. If you don't do the whole thing, the plant won't have power. If you eat the fresh plant nothing will happen – it doesn't have power. I learned to prepare it from my father." (Smith et al., 1996).

It is likely that the compound responsible for the psychoactive properties of 'kougoed' is an alkaloid which was first isolated from *Scelletium tortuosum* (Meiring, 1898). It was only in 1914 that Zwicky, after isolating the alkaloid from *S. expansum* and *S. tortuosum*, assigned the name mesembrin (now mesembrine) to these alkaloids.

Zwicky's findings were based on general alkaloid tests and thus did not necessarily indicate the presence of only the mesembrine alkaloids. It should also be noted that levels of secondary plant metabolites including the alkaloids are strongly influenced by season, growing conditions, and age of plants. Turnover and degradation of plant alkaloids and seasonal fluctuations in levels have been documented in many cases (Barz & Koster, 1981). This fluctuation, if it exists in *Scelletium*, may well account for van der Stel's observation of 1685 "when we came to the Coperbergh in October, it was being gathered" (Waterhouse, 1932), as it may be that at this time of year the alkaloid levels are at their highest.

October is significant in the reproductive cycle of *Scelletium* as, it is at this time of year that fruit production occurs, based on our observations of plants growing in cultivation. Alkaloids have often been implicated as an antiherbivory device (Henry, 1949), and it is possible that *Scelletium* produces more alkaloid in the aerial portions of the plant at this time of year. Jeffs et al. (1971) have reported that the alkaloid concentrations were greatest in the woody stems of *Scelletium* plants and decreased in the order root >> green stem > leaf. However, it should be noted that the time of harvest of

these plants was not given and could well change with the season.

One of the objectives of the present study was to develop a technique for extracting alkaloids from gram quantities of dried plant material, and then to apply this technique to obtain semi-quantitative data on the distribution of mesembrine alkaloids in the nine genera and twenty species of Mesembryanthemaceae available. This would provide evidence as to whether the mesembrine alkaloids are restricted to the genus *Scelletium*, or are also present in other genera of the Mesembryanthemaceae as has been indicated (Watt & Breyer-Brandwijk, 1962).

Several references allude to the psychoactive properties of 'kougoed' (e.g. Laidler, 1928; Waterhouse, 1932; Waterhouse et al., 1979), as well as a more direct use of *Scelletium* plants. Non-fermented approaches to *Scelletium* use include the preparation of tinctures, direct chewing of plants, and the juices from masticated material being spat into the mouths of unsettled infants (Smith et al., 1996). In view of these two approaches to plant use it was decided to simulate the fermentation process in order to assess its possible influence on plant alkaloid levels.

MATERIALS AND METHODS

Plant Materials

With the exception of *Scelletium tortuosum*, which was grown from seed, plants used in this study were obtained from a local nursery and potted out under 40% shade cloth in the University of Natal, Botany Department gardens. All plants were between three and four years old, and were harvested for extraction during the winter season before flowering.

Extraction Technique

Alkaloids were extracted from a total of 75 g dried plant material. Three lots of 25 g each representing root, stem and leaf material were extracted with 200 ml 95% ethanol for 12 h in a Soxhlet apparatus. The ethanol extract was taken to dryness on a rotary evaporator and then redissolved in 20 ml 2N hydrochloric acid. This aqueous acid solution was partitioned against three washes of diethyl ether (150 ml) to remove any fatty material and pigments. The resultant aqueous solution was applied to columns packed with 60 ml Extrelut (E. Merck, Darmstadt). After 20 min an elution was carried out using 40 ml dichloromethane: isopropanol (85:15) and the eluate discarded. The column

was then basified using ammonia gas and the alkaloids eluted with 40 ml dichloromethane:isopropanol (85:15). Due to the exceptionally low levels of mesembrine alkaloids in all species except *Sceletium tortuosum* eluates representing the three 25 g lots were collected and pooled. The solvent was removed on a rotary evaporator to 2 ml which was then subjected to column chromatography on silica gel slurried in dichloromethane (column 17 × 2 cm, Sigel Merck Art. 7733). Six successive elutions of 35 ml each were carried out with solvents of increasing polarity (dichloromethane, ethyl acetate, acetone, acetonitrile, methanol and acetic acid). These were concentrated to 2 ml and analyzed by injection of 1 μ l samples onto a GC fitted with a NPD.

Thin Layer Chromatography

To test for the presence of alkaloids, representative samples of the crude extract were streaked onto Merck 5 × 20 cm silica gel plates and run twice in dichloromethane:methanol (3:1). Identification of the alkaloids was carried out using Dragendorff spray reagent.

Gas Chromatography and Mass Spectrometry

GC was carried out using a Varian 3300 instrument fitted with a Nitrogen-Phosphorous specific detector (NPD) and a 25 m, BP5 bonded column (SGE, Australia) of 0.53 mm internal diameter and 1 μ m film thickness. The column program was from 230°C to 260°C at 1°C per min. The detector was set at 350°C. The chromatographic data obtained by the GC was recorded on a Hewlett Packard 3395 integrator.

Gas Chromatography/Mass Spectrometry (GC/MS) was carried out using a Finnigan Mat ITS40 instrument fitted with a 30 m fused silica DB-5 capillary column of 0.25 mm internal diameter (J and W Scientific, Inc.). 1 μ l samples were injected onto the GC/MS using the same column temperature program as for GC/NPD work. Structures of the compounds were elucidated based on their respective mass spectra as published by Martin et al. (1976).

Application of Technique to Other Species

Once the retention times and spectra for the mesembrine alkaloids had been confirmed for the *Sceletium* material, their presence in other species was determined by the retention times of peaks using the GC with NPD. Where appropriate, peak identity was confirmed using GC/MS. Some compounds were found in the other genera which had shorter retention times than

the mesembrine alkaloids and which gave a response on the NPD. These peaks were also analyzed by GC/MS.

To correct for day-to-day variation in retention times on the NPD, a *Sceletium* sample was run before and after each set of samples.

Kougoed Production

Three *Sceletium* plants were harvested, washed to remove excess soil and crushed, using a mortar and pestle, with a small amount of soil (roughly 1 g) in order to simulate the soil that would be incorporated by crushing between two stones as was reportedly done by the KhoiSan. The resulting mass of ground plant material was placed in a plastic bag which was squeezed to remove entrapped air and sealed to render it air-tight. Two controls were used: three *Sceletium* plants harvested and dried at 80°C without crushing; three *Sceletium* plants were harvested, crushed and then immediately dried at 80°C. The "fermented" sample was dried at 80°C prior to alkaloid extraction. In a separate experiment to investigate the role of enzymatic processes and the effect of drying crushed plant material at 80°C, three plants were crushed in liquid nitrogen. The crushed material was thoroughly mixed to eliminate any plant to plant variation. Three samples, each of 3 g, of this homogenous mass was subjected to the following treatments; (1) resuspended in water and dried at 80°C, (2) resuspended in water and allow to 'ferment' in a plastic bag for eight days; and (3) boiled in 80% ethanol and extracted immediately.

RESULTS

Thin Layer Chromatography

Thin layer chromatography of the crude extracts resulted in a number of Dragendorff-positive bands, some of which corresponded to the R_f values for the mesembrine alkaloids found in the literature. Where levels of the alkaloids were low it was not possible to consistently recover them for further analyses; consequently, a column chromatography method was employed.

Column Chromatography

Column chromatography was used to fractionate the *Sceletium* alkaloid extract, prepared by the Extrelut column. Analysing each fraction by GC it was found that the acetone fraction eluted three mesembrine alkaloids while the dichloromethane and ethyl acetate fractions

were devoid of these alkaloids. A small amount of the mesembrine alkaloid was found to be eluted by the acetonitrile fraction but none eluted in the methanol or acetic acid fractions. All alkaloids were eluted with acetone when the elution volume was changed from 35 to 70 ml.

Mass Spectrometry

Acetone was found to elute the mesembrine alkaloids from a silica gel column, which appeared as three distinct peaks by GC (Fig. 1). These were identified as (1) 4'-*O*-demethylmesembrenol: retention time 11.5 min; $[M]^+$ 275 (55%), $[M-1]^+$ 274 (33%), $[M-Me]^+$ 260 (6%), 232 (15%), 219 (28%), 218 (35%), 204 (100%), 137 (9%), 110 (8%), 96 (92%), 70 (98%); (2) mesembrine retention time 12.0 min; $[M]^+$ 289 (66%), $[M-1]^+$ 288 (36%), $[M-Me]^+$ 274 (9%), 246 (8%), 232 (19%), 219 (48%), 218 (92%), 204 (48%), 151 (11%), 110 (4%), 96 (100%), 70 (87%); (3) mesembrenone: reten-

tion time 12.5 min; $[M]^+$ 287 (21%), $[M-1]^+$ 286 (4%), $[M-Me]^+$ 272 (2%), 259 (4%), 258 (5%), 244 (2%), 230 (3%), 219 (7%), 218 (2%), 204 (2%), 149 (2%), 110 (< 1%), 96 (2%), 70 (100%).

Alkaloid Levels in *Scaletium* and Other Genera

Based on retention times, a number of other genera appeared to have the mesembrine alkaloids, although they were at very low levels. In addition, genera other than *Scaletium* contain a number of compounds with retention times shorter than those for the mesembrine alkaloids (typically 7.2; 8.1; 8.7 and 9.1 min). Since no standards were available for quantitative analysis, the NPD response and peak area counts from the integrator were used to provide semi-quantitative data for the levels of the peaks observed.

Using this approach, the seven species of the genus *Delosperma*, which were examined showed negligible or non-detectable levels of the mesembrine alkaloids;

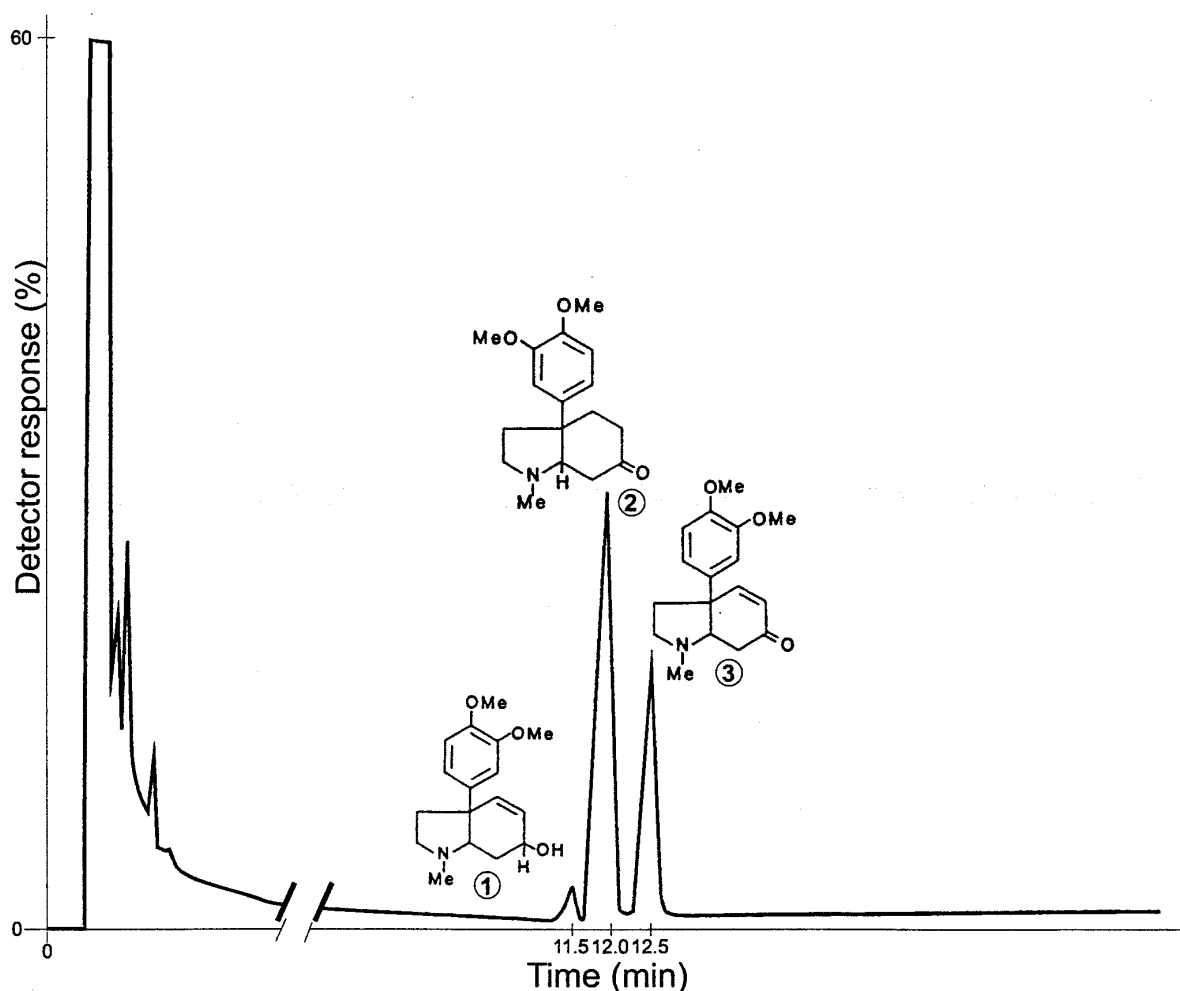


Fig. 1. Chromatogram of *Scaletium tortuosum* showing three mesembrine alkaloid peaks; 1. 4'-*O*-demethylmesembrenol, 2. mesembrine and 3. mesembrenone.

a notable exception being *Delosperma minimum* which contained 15% of the level of 4'-*O*-demethylmesembrenol seen in *Sceletium* (Table 1). One striking characteristic in chromatograms of this genus was the recurring peaks with retention times of 7.2, 8.1, 8.7 and 9.2 min. Of the five *Lampranthus* species tested, only *L. aureus* and *L. spectabilis* yielded mesembrenol, while all the other *Lampranthus* species investigated appeared to contain mesembrenone, but all at very low levels (Table 1). A peak with retention time 8.1 min was only found in *L. aureus* (Table 1). A large peak with a retention time of 8.7 min was seen in *L. aureus*, which was the only compound that quantitatively equalled the levels of the putative mesembrine peak. It was also present in *L. roseus*, *L. spectabilis* and *L. blandus*, but at very low levels (Table 1). A peak at 7.2 min was only found in *L. spectabilis*, *L. blandus* and *L. coccineus* and *L. deltoides*, and then only at very low levels (Table 1). The two species of *Drosanthemum* investigated contained 4'-*O*-demethylmesembrenol and in *D. hispidum* the level of 4'-*O*-demethylmesembrenol was the fourth highest of all the plants tested (Table 1); mesembrenone levels were approximately half that of demethylmesembrenol. *Aptenia cordifolia* showed the second highest levels of mesembrine and of demethylmesembrenol of the plants studied (Table 1). Unidentified minor peaks were also observed at 9.2, 8.7 and 7.2 min. *Bergeranthus scapiger* was found to have low levels of both demethylmesembrenol and mesem-

brenone (Table 1). *Glottiphyllum longum*, and *Ruschia lineolata* showed a complete absence of the mesembrine alkaloids and insignificant levels of nitrogen-containing compounds at 7.2 min (Table 1).

Mass Spectra for Other Compounds

The mass spectrum obtained for the compound eliciting a response on the NPD at retention times 9.2 min in *Delosperma potsii* (with the exception of two very minor fragments at *m/z* 319 and *m/z* 347) were identical to mass spectrum of the peak at 8.1 min in *D. pruinatum*. The peak in *Lampranthus aureus* with a retention time of 8.7 min also yielded similar fragments. These compounds appear to contain an indole ring, with diagnostic ions at *m/e* 57; 71; 149 and 167 corresponding to known fragments (Martin et al., 1976). The mass spectrum for the peak at 8.7 min in *Lampranthus aureus* also contains fragments some of which are the same as those in the mass spectra for the peaks with retention times 8.1 and 9.2 min.

Relative Levels of the Alkaloids in *Sceletium* and 'Kougoed'

The fermenting *Sceletium* sample was found to be foul smelling and possessed observable fungal growth. Comparison of the integrator counts for the alkaloid extract of the fermented product yielded marked variations in peak sizes and ratios compared to that of the control. While the peak of 4'-*O*-demethylmesembrenol

Table 1. Relative levels of the alkaloids found in Mesembryanthemaceae species screened represented as a percentage of the mesembrine peak found in *Sceletium tortuosum*.

Species studied	Retention time (min)						
	7.2	8.1	8.7	9.2	11.5	12	12.5
<i>Sceletium tortuosum</i> (L.) N.E.Br.	—	0.2	—	0.4	8.1	100	69.4
<i>Aptenia cordifolia</i> (L.f.) Schwant.	0.3	—	0.2	4.6	14.4	9.7	—
<i>Bergeranthus scapiger</i> (Haw.) N.E.Br.	—	—	—	—	0.9	—	0.5
<i>Ruschia lineolata</i> (Haw.) Schwant.	0.4	—	—	—	—	—	—
<i>Glottiphyllum longum</i> (Haw.) N.E.Br. var. <i>longum</i>	1.9	—	—	—	—	—	—
<i>Drosanthemum hispidum</i> (L.) Schwant. var. <i>hispidum</i>	—	—	—	—	2.5	—	1.2
<i>D. bicolor</i> L.Bol.	—	—	—	0.1	1.1	—	0.5
<i>Delosperma minimum</i> Lavis	—	0.9	—	0.9	15	—	0.8
<i>D. pruinatum</i> (Thunb.) J. Ingram	1.9	8.2	—	—	4.8	6.2	6.5
<i>D. potsii</i> (L.Bol.) L.Bol.	2.2	3.9	1.5	31.5	0.8	0.1	0.4
<i>D. cooperi</i> (Hook.f.) L.Bol. forma <i>cooperi</i>	—	—	8.0	—	0.8	—	0.2
<i>D. rogersii</i> (Schoenl. & Berger) L.Bol. var. <i>rogersii</i>	—	—	0.7	0.2	0.8	—	—
<i>D. lebombense</i> (L.Bol.) Lavis	1.2	—	0.6	—	—	—	0.2
<i>D. obtusum</i> L.Bol.	—	—	—	—	0.2	—	—
<i>Lampranthus aureus</i> (L.) N.E.Br.	—	0.5	82	—	1.3	—	1.2
<i>L. roseus</i> (Willd.) Schwant.	—	—	1.1	—	—	—	0.2
<i>L. blandus</i>	0.2	—	0.2	—	—	—	0.1
<i>L. spectabilis</i> (Haw.) N.E.Br. subsp. <i>spectabilis</i>	0.1	—	0.2	—	0.1	—	0.2
<i>L. deltoides</i> (L.) Wijnands	0.7	—	—	—	—	—	—
<i>L. coccineus</i> (Haw.) N.E.Br.	0.6	—	—	—	—	—	0.2
<i>Oscularia deltoides</i>	0.7	—	—	—	—	—	—

had almost completely diminished, the peak for mesembrine was halved, and the mesembrenone peak had doubled (Fig. 2). A similar pattern was observed for a sample which was crushed and immediately dried. When examining the chromatograms from the second experiment in which any enzymatic reactions were stopped by freezing in liquid nitrogen, it was found that material dried at 80°C had a similar alkaloid ratio and concentration as the fermented material while the sample that was boiled in ethanol and immediately extracted had an alkaloid ratio and concentration similar to that of uncrushed plant material.

DISCUSSION

Allowing for the fact that plant alkaloid levels may vary as a result of a number of different endogenous and

exogenous factors, it is nonetheless striking that the highest alkaloid levels were found in material of *Sceletium*. *Aptenia* was found to contain mesembrine alkaloids in significant concentrations, relative to the other taxa examined, although these were only 13.6% of those seen in *Sceletium*. The ethnopharmacological significance of very low alkaloid levels in the majority of genera examined is clear: it would be almost impossible to achieve any pharmacological response from genera other than *Sceletium*, since approximately 60–80 kg of plant material (fresh weight) would have to be ingested to achieve a response. It is clear also that since *Aptenia* lacks mesembrenone its 'fermentation' would fail to produce elevated levels of this alkaloid.

'Kougoed', when prepared by the contemporary plastic bag method (Smith et al., 1996) was found to contain the mesembrine alkaloids at levels and ratios substantially different from those of unfermented mate-

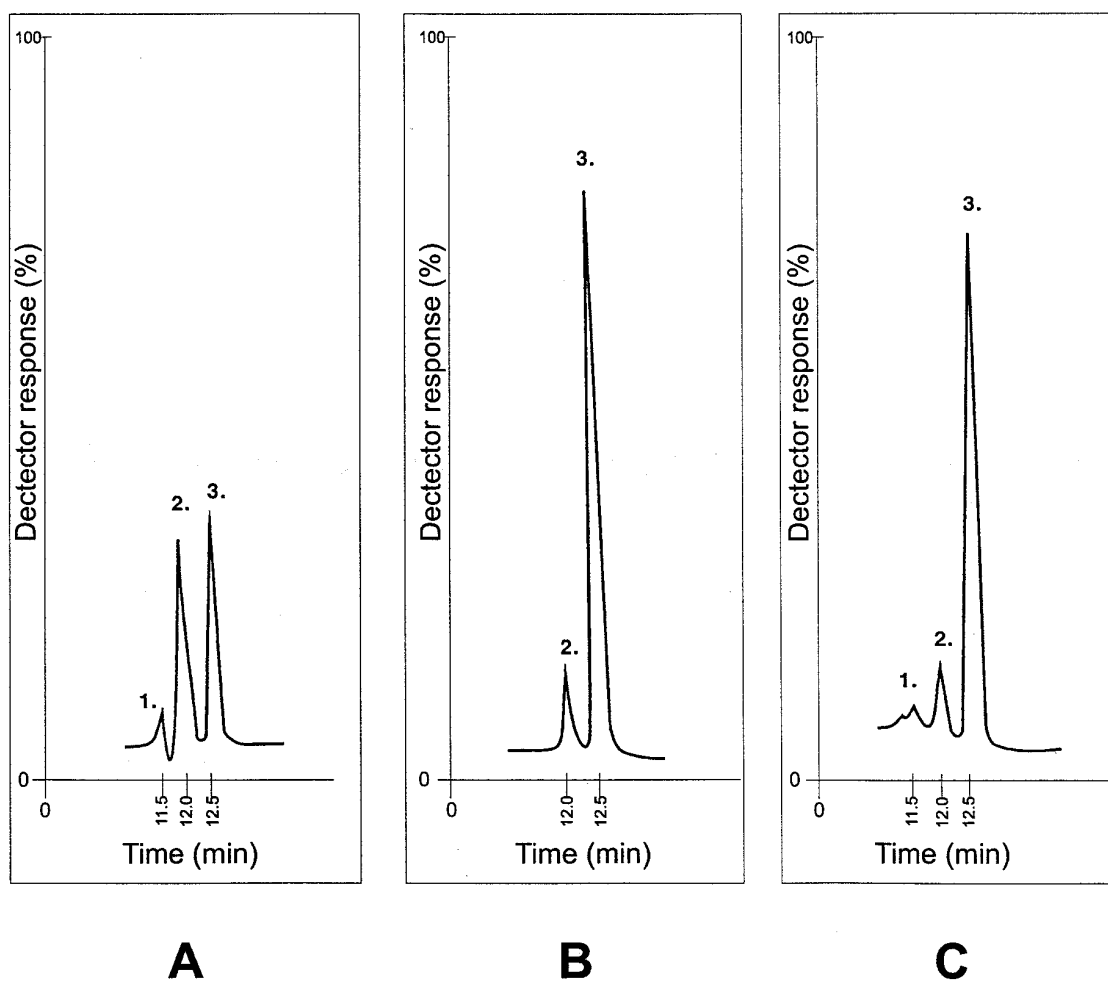


Fig. 2. Chromatographic profiles of *Sceletium tortuosum* extracts showing the different levels of 1. 4'-*O*-demethylmesembrenol, 2. mesembrine and 3. mesembrenone for: (A) uncrushed plant material dried at 80°C, (B) crushed and fermented plant material and (C) crushed plant material dried at 80°C.

rial. In addition, a marked decline was seen in 4'-*O*-demethylmesembrenol and mesembrine levels, whilst mesembrenone levels increased significantly. When fresh plant material of *Sceletium* was crushed, and immediately dried at 80°C thereafter, a similar alkaloid profile to that of the 'kougoed' sample was observed both for the alkaloid levels and ratios which were greatly changed over those of non-crushed, oven-dried material. Thus, the folk method of preparation appears to have two, as yet unexplained, principles at work.

It is possible that, on crushing, enzymatic reactions may take place following cellular decompartmentation, these reactions may explain the modification of the alkaloid ratio and concentration that is observed in crushed plant material dried at 80°C; a temperature at which these reactions may be greatly amplified. In the second experiment these were suspended by crushing the plants in liquid nitrogen. The enzymatic reactions would be resumed with resuspension of the plant material in water but be eliminated by boiling in ethanol and immediate extraction. From the results of this experiment it would seem that the essential step in the production of 'kougoed' may not entirely revolve around 'fermentation' but that the crushing of the plant material and consequently the mixing of cellular material may be equally essential.

It can therefore be concluded that the traditional use of *Sceletium* over other Mesembryanthemaceae, and its method of preparation as 'kougoed' and the alternative quick preparation method (Smith et al., 1996) of heating crushed material under a fire have a rational pharmacological basis, perhaps evolved over many generations through trial-and-error experimentation.

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