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ANTINEOPLASTIC AGENTS FROM HIGHER PLANTS: APPLICATION OF TANDEM MASS SPECTROMETRY TO XANTHONES FROM *PSOROSPERMUM FEBRIFUGUM*

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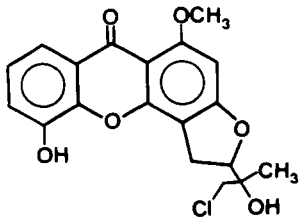
ABSTRACT.—*Psorospermum febrifugum* was examined using tandem ms for the presence of 3′,4′-deoxypsorospermin-4′-chloro-3′-ol and psorospermin. Collision-induced dissociation at both high and low energy is used to bring about fragmentation. Daughter spectra were interpreted to reveal characteristic fragmentations for both compounds. Examination of the authentic compounds, various extracts of the plant root, and the plant root material itself established that psorospermin occurs naturally in the plant. The data also suggest the presence of the chlorohydrin in the root, as well as establishing its presence in the root extracts. The detection of neutral compounds in complex mixtures by tandem ms is facilitated if comparisons can be made between spectra resulting from different methods of ionization and excitation.

Previous studies of *Psorospermum febrifugum* Sprach. (Guttiferae) have failed to yield conclusive evidence as to whether 3′,4′-deoxypsorospermin-4′-chloro-3′-ol (**1**) is a natural product or an artifact due to the isolation procedure (1,2). In view of the biological importance of this type of compound and the success of ms/ms (tandem ms) in the analysis of mixtures (3-6), this technique was chosen in an attempt to resolve the problem. Psorospermin (**2**), a related xanthone also derived from the title species, was simultaneously studied. The data obtained establish that both **1** and **2** occur in crude alcoholic root extracts (experiments done without using chlorine-containing solvents) and that **2** is present as such in the plant. Although the data suggest the possibility that the chlorohydrin exists in the root material, there is also evidence for the presence of another chlorine-containing compound.



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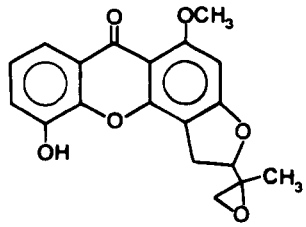

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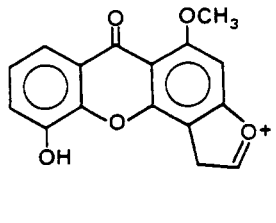

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Tandem ms has been extensively employed in directly characterizing constituents of complex mixtures, such as drugs or naturally occurring compounds in biological fluids and tissues (7-10). A major portion of this work has dealt with acidic or basic compounds. Compounds such as alkaloids in plant extracts (11) or carboxylic acids generated from oxidative degradation of coal (12) can be selectively ionized from mixtures, thus simplifying the task of characterization. Previous work on the application of ms/ms to neutral constituents of natural products has revealed this to be more demanding than characterization of highly polar compounds (13,14).

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In order to increase the specificity of the tandem mass spectrometric analysis, two forms of the ionized target molecules have been prepared using different ionization techniques. In addition, their spectra have been recorded in two different energy regimes. High and low energy collisions can yield substantially different daughter ion spectra due chiefly to differences in the distributions of internal energy acquired by the parent ion in collisions under these conditions (15).

EXPERIMENTAL

High collision energy daughter spectra were obtained using a mass-analyzed ion kinetic energy spectrometer (MIKES) (16). Parent ions were selected with the magnetic sector. Collision with argon at 7000 eV collision energy and a nominal pressure of 2×10^{-5} torr was used to induce fragmentation. Daughter ions were mass-analyzed using the electrostatic sector. The broad peaks of MIKE spectra are caused by the kinetic energy release that occurs during fragmentation.

Low collision energy spectra were obtained on a Finnigan triple-stage-quadrupole (TSQ) mass spectrometer (17). The parent ions were selected using the first quadrupole and collided at 20 eV with argon at 2×10^{-3} torr in the second (Rf-only) quadrupole. Daughter ions were analyzed using the third quadrupole. The variation in the fragment ion peak intensities relative to the parent ion is most likely due to fluctuations in the collision gas pressure between experiments.

In each experiment, two types of ionization method were used. Chemical ionization (CI) using methane as the reagent gas was used to generate protonated molecules, $(M+H)^+$. Charge exchange with argon (MIKES) or electron impact (TSQ) was used to generate molecular ions, $M^{+\cdot}$. Source pressures were 115 mtorr (MIKES) and 350 mtorr (TSQ) for charge exchange and chemical ionization. Probe temperatures were ca. 250° and source temperatures ca. 150° for both instruments.

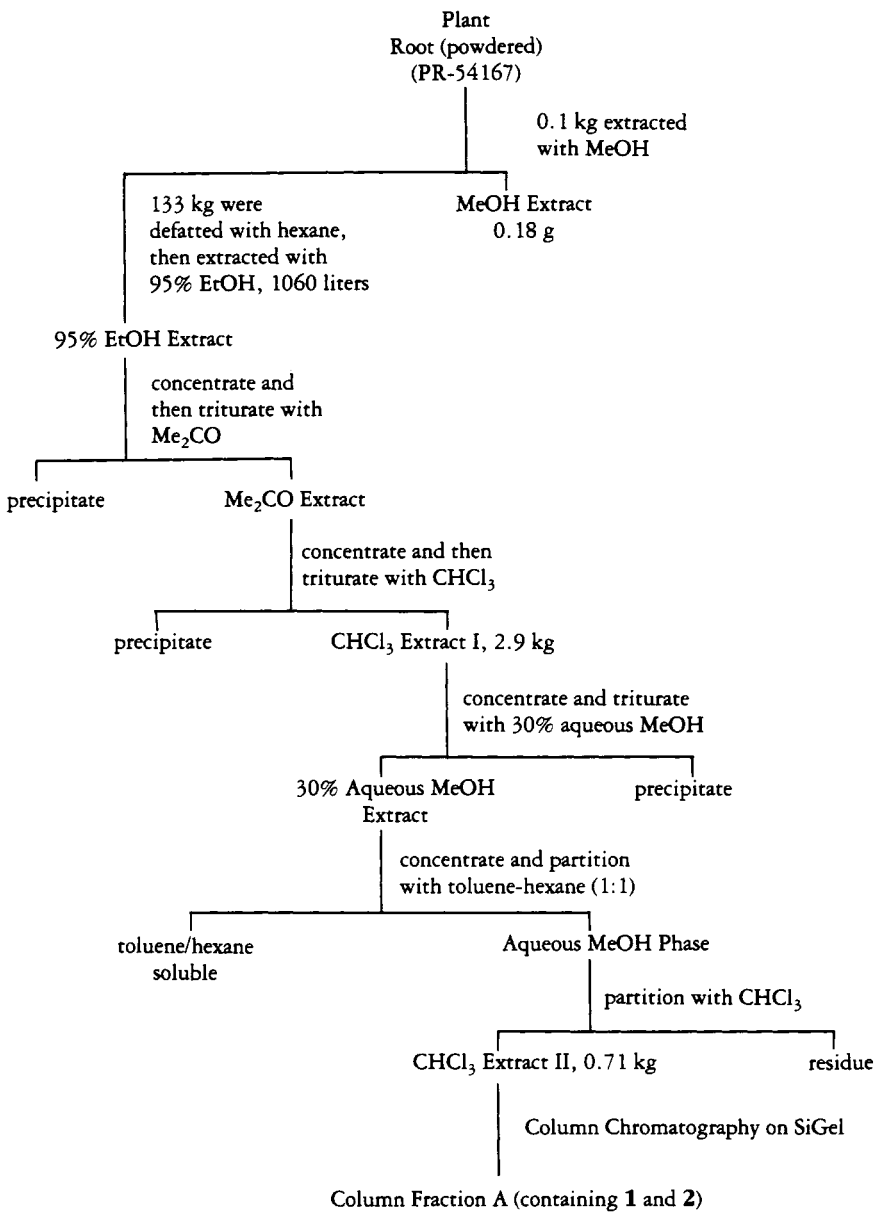
Psorospermin (**2**) and the chlorohydrin **1** were isolated from the plant root material by the procedure outlined in Scheme 1. The large scale extraction and fractionation was carried out at Polysciences Laboratory, Warrington, Pennsylvania, and this procedure generated all of the fractions leading to the chromatographic step. The small scale MeOH extract was used to monitor the possible production of artifacts in the large-scale procedure. The roots of *P. febrifugum* were collected in Tanzania in 1978 by Mr. Leonard Mwasumbi of the University of Dar-es-Salam. Specimens were authenticated at the Economic Botany Laboratory, Beltsville Agricultural Research Center, Beltsville, Maryland, where a voucher specimen is on deposit.

RESULTS AND DISCUSSION

Psorospermin and its chlorohydrin give characteristic daughter spectra when either the molecular ions ($M^{+\cdot}$) or the protonated molecules $(M+H)^+$ are dissociated upon collision. Such spectra characterize the precursor ion (and hence the neutral molecule from which it is derived) via the masses and abundances of the fragments (18-20). Daughter spectra should be useful in identifying xanthones in mixtures, and interferences from other mixture constituents should be minimized or recognizable by obtaining both high and low collision energy data for both $M^{+\cdot}$ and $(M+H)^+$. The following presentation covers 3′,4′-deoxypsorospermin-4′-chloro-3′-ol data before those of psorospermin are considered.

The daughter spectrum of protonated authentic 3′,4′-deoxypsorospermin-4′-chloro-3′-ol obtained at high collision energy is shown as the upper spectrum in Figure 1. The spectrum shows the rather poor mass resolution inherent in using a kinetic energy analyzer for mass analysis. Nevertheless, the major reactions observed correlate with the structural features of **1**. The base peak is due to dehydration, while losses of $CH_3\cdot$ and CH_4 give rise to the other abundant ions in the high mass region. These reactions are consistent with the presence of hydroxyl and methyl groups in the side chain. The ion at *m/z* 283 is rationalized as the stabilized oxonium ion (**3**), generated by the loss of the entire side chain, while the ion at *m/z* 282 is due to loss of the side chain with a hydrogen atom. Formal loss of a methyl group from these ions leads to *m/z* 268 and 267.

From Figure 1, it appears that the authentic chlorohydrin (**1**), or a compound that gives a similar spectrum, occurs in the root. Parallel features in the daughter spectra of



SCHEME 1. Extraction procedure for the xanthones from the plant root material of *Psorospermum febrifugum*.

an authentic compound with that of the same molecular weight in a complex mixture have been shown to verify the presence of that component in the mixture (11). For the chlorohydrin, note the similarities in the peaks and intensities at *m/z* 362, 360, 359, 283, 282, and 267 in the authentic compound and the plant root material spectra. The additional peaks in the spectrum of the plant root material probably arise from the matrix or are possibly due to an additional component at this mass. However, the spectra of the chromatographic fractions (not reproduced) fail to show the characteristic fragmentation pattern of the authentic chlorohydrin. This behavior is in contrast to that observed for the root material. While the data of Figure 1 suggest the presence of **1** or a

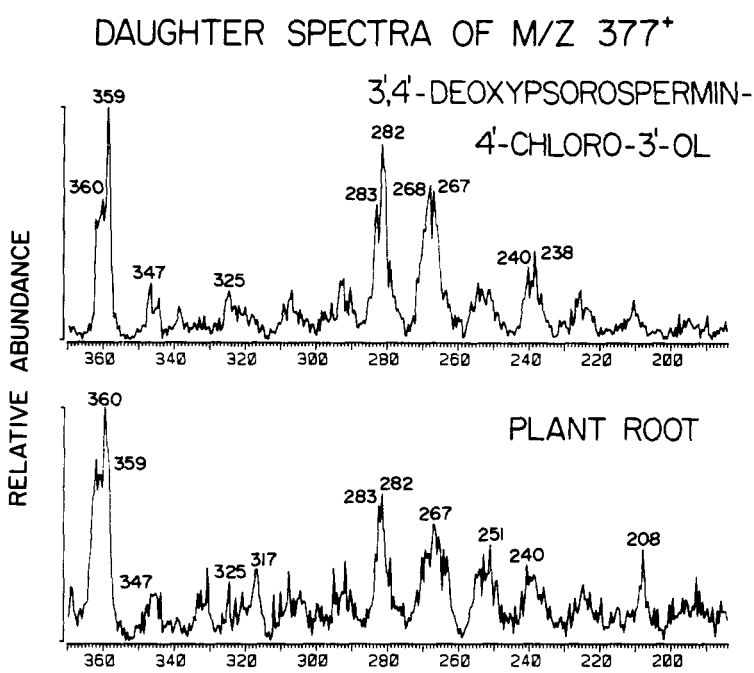


FIGURE 1. Ms/ms daughter spectra of protonated 3',4'-deoxypsorospermin-4'-chloro-3'-ol (*m/z* 377) and the plant root material at *m/z* 377 recorded at 7000 eV collision energy by ESA scanning.

closely related compound, data in a different energy regime were needed to give complementary results and allow firm conclusions to be drawn.

The daughter spectrum of protonated authentic 3',4'-deoxypsorospermin-4'-chloro-3'-ol recorded at low collision energy, shown as part of Figure 2, is quite different from that recorded at high energy. The major fragmentations following collision at low energy are rationalized in Scheme 2. As in the high collision energy spectrum, many of the fragmentations involve the side chain. These can be rationalized as leading to particularly stable fragment ions. Note that sequential loss of HCl, H_2O, and C_2H_4 can lead to a pyrylium ion, *m/z* 295, with side-chain-carbon insertion into the five-membered ring, while loss of a carbon atom from the five-membered ring together with the side chain leads to the stable quinone-type ion, *m/z* 271. The ions at *m/z* 309, 299, and 285 have lost part or all of the side chain to give stable products that include the original ring system. Many of the reactions in Scheme 2 probably proceed via loss of HCl rather than in one step from the protonated molecule. The importance of the chlorine in directing the fragmentation behavior of the protonated molecule is further indicated by comparison with the types of reactions occurring in psorospermin (see below). The spectra for both compounds have some similar fragment ions containing only the ring system, independent of side-chain substituents. Comparison of the high and low energy daughter spectra of the authentic chlorohydrin reveal evident differences in the masses of the major ions and their relative intensities even though many of the same bonds are cleaved. These dissimilarities, which are striking but not unprecedented (21-23), are probably associated with differences in energy deposited upon low and high energy collisions. We do not exclude the possibility of collisions in the keV range resulting in excited states of the ion, which display different unimolecular chemistry from that of the ground state (24-26).

The triple quadrupole daughter spectra of *m/z* 377 in the plant root, the crude EtOH and MeOH extracts, and various chromatographic fractions appear in Figure 2.

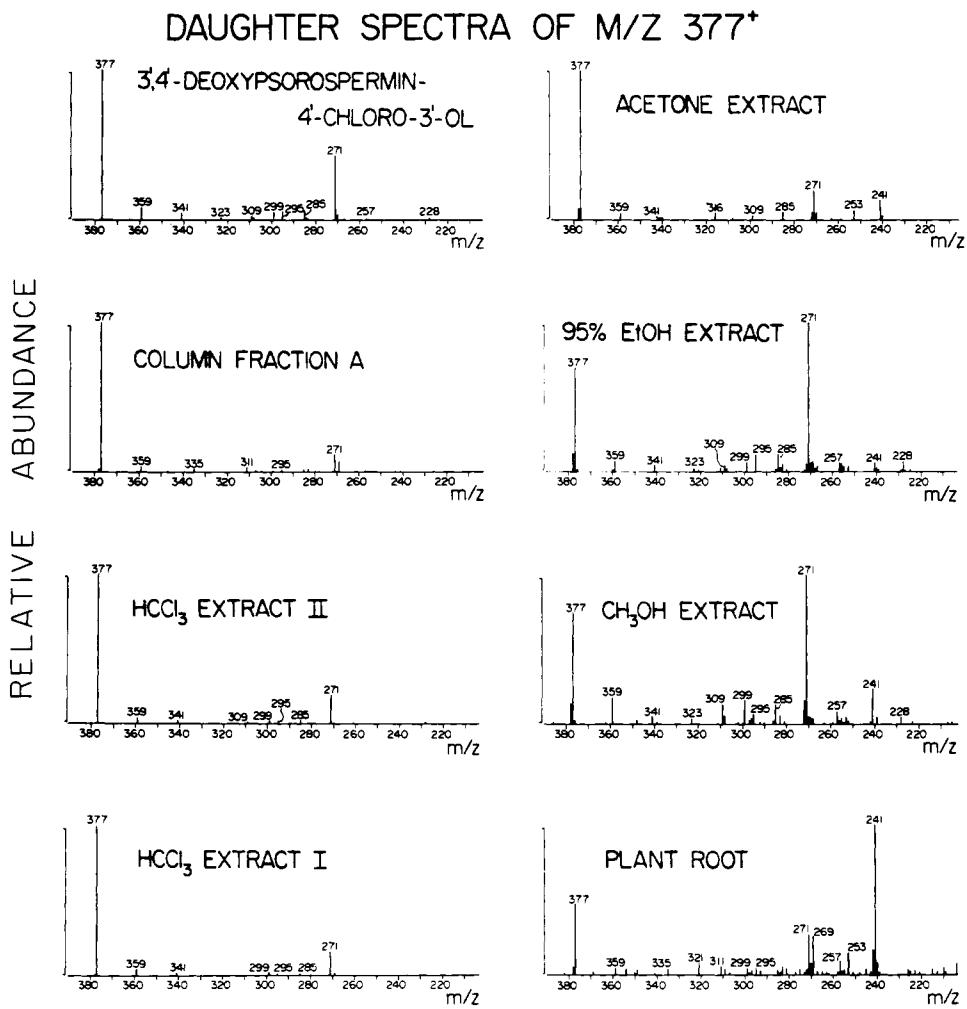
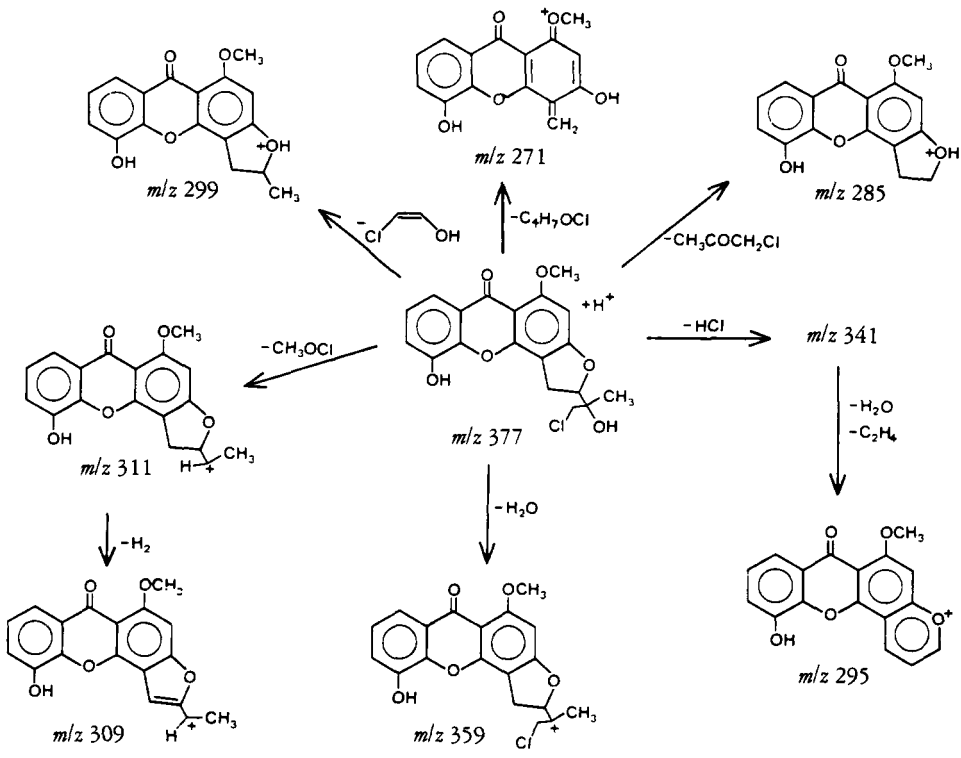


FIGURE 2. Ms/ms daughter spectra of protonated 3',4'-deoxypsorospermin-4'-chloro-3'-ol (*m/z* 377), samples of the extracted root material after various degrees of purification, and the plant root material at *m/z* 377 recorded at 20 eV collision energy by quadrupole scanning.

These data are of much higher quality than the corresponding high energy spectra, and they suggest that the chlorohydrin is not the most abundant compound at protonated mass 377, which is released directly from the root into the mass spectrometer. They also show that (a) the major constituent liberated from the root material upon heating of the direct insertion probe, which gives a characteristic fragment ion at *m/z* 241, is also present in the MeOH and EtOH extracts of the root, but is gradually removed as the sample is purified chromatographically, and that (b) the crude alcoholic extracts contain the isolated chlorohydrin as the major constituent at mass 377 (after protonation). A plausible explanation of these data is that an isomer of the chlorohydrin is the major constituent liberated directly from the root but that alcoholic extraction yields both this compound and the chlorohydrin.

Daughter spectra of the molecular ion ($M^{+\cdot}$, *m/z* 376) of the chlorohydrin appear in Figure 3 (high energy collisions) and Figure 4 (low energy collisions). The high energy spectrum shows the loss of H_2O and CH_3 from the side chain, resulting in the fragment ions *m/z* 358 and 361. Most of the ions occurring in the lower mass region result from further fragmentation of the side chain. The most obvious difference in the high energy



SCHEME 2. Main fragmentations of protonated 3',4'-deoxypsorospermin-4'-chloro-3'-ol induced by 20 eV collisions.

spectra of the $(M+H)^+$ ion and the $M^{+\cdot}$ ion is the presence of the intense ion at *m/z* 278 appearing in the daughter spectrum of the $M^{+\cdot}$ ion.

The low energy daughter spectrum of the molecular ion $M^{+\cdot}$ shows more fragmentation than the spectrum of the corresponding protonated molecule. The molecular ion readily loses a hydrogen atom resulting in the ion at *m/z* 375, which subsequently loses 28 daltons, possibly the carbonyl group from the xanthone structure, to form the ion at *m/z* 347. Carbonyl losses also appear to occur in the fragmentation of other ions in this spectrum as is shown in Scheme 3. The molecular ion losses the hydroxy substituent from the side chain as well as the entire side chain. Each of these ions loses 28 daltons to

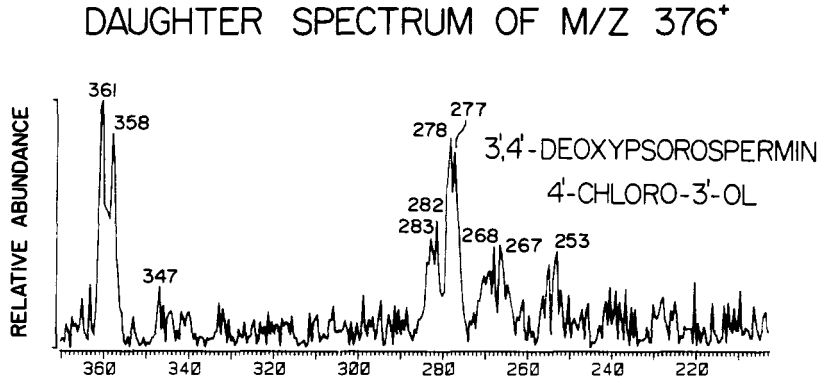


FIGURE 3. Ms/ms daughter spectrum of the molecular ion (*m/z* 376) of 3',4'-deoxypsorospermin-4'-chloro-3'-ol generated by charge exchange with argon and recorded at 7000 eV by ESA scanning.

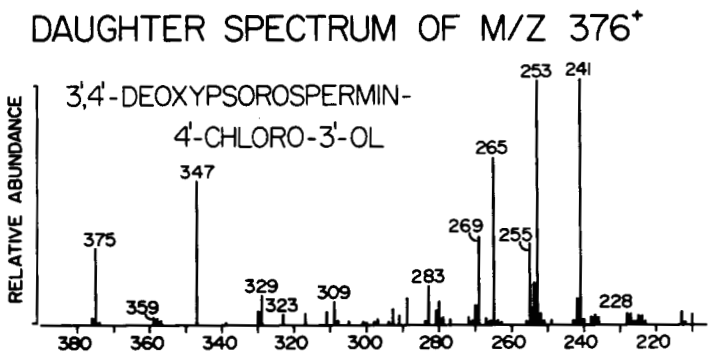
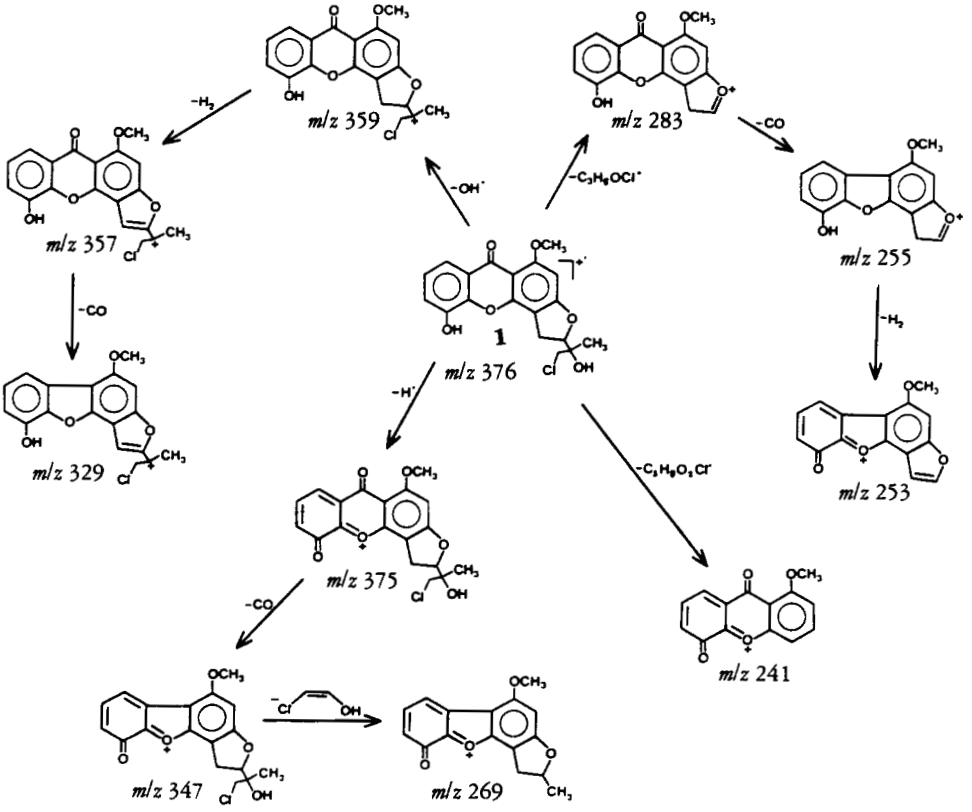


FIGURE 4. Ms/ms daughter spectrum of the molecular ion (*m/z* 376) of 3',4'-deoxypsorospermin-4'-chloro-3'-ol generated by electron impact and recorded at 20 eV by quadrupole scanning.

yield the ions at *m/z* 329 and 255, respectively. The intense ion at *m/z* 241 arises from the loss of the five-membered ring leaving the xanthone structure intact. Note that Scheme 3 rationalizes the postulated fragmentation behavior of this compound. A study of unsubstituted xanthone has shown the loss of CO from the middle ring to be the most facile reaction (27). When the molecule bears substituents, the loss of the substituent occurs before fragmentation of the xanthone structure (27). This has been shown to be the case for simple methoxy derivatives (27-29), as well as more complex compounds (30,31). By extrapolation from these earlier studies, fragmentation of the



SCHEME 3. Main fragmentations of 3',4'-deoxypsorospermin-4'-chloro-3'-ol ($M^{+\cdot}$) induced by 20 eV collisions.

side chain in 3′,4′-deoxypsorospermin-4′-chloro-3′-ol in the ms/ms experiment is expected, followed by CO elimination from the xanthone skeleton.

Data on the plant root and extracts were recorded after employing electron impact and charge exchange to generate the molecular ion. Both the MIKE and triple quadrupole spectra were consistent with the interpretations reached regarding the chlorohydrin from chemical ionization. However, the data were of much lower quality, and they are therefore not reproduced.

Daughter spectra were taken with the low energy triple quadrupole instrument for the naturally occurring ^{37}Cl isotope of 3′,4′-deoxypsorospermin-4′-chloro-3′-ol at *m/z* 379 to seek additional information on the identity of the component in the plant material that appears to be related to the chlorohydrin. The daughter spectrum for the heavier isotope of the protonated authentic compound is compared with that of the ion derived from plant root material in Figure 5. The authentic chlorohydrin gives a spectrum identical to that of the ^{35}Cl-compound (Figure 2) with the exceptions of the protonated molecular ion $(M+H)^+$ and its dehydration product $(M+H-H_2O)^+$, both of which show the appropriate isotopic mass shift. Fragment ions that have lost chlorine are unshifted, and this information helps confirm the assignments made in Scheme 2. The daughter spectrum of *m/z* 379 derived from the plant root material has fragment ion peaks which correspond to those in the plant root spectrum of *m/z* 377. Note the reappearance of the ion at *m/z* 241 and the pair of peaks at *m/z* 269 and 271. The ion at *m/z* 361 in the daughter spectrum of the ^{37}Cl-isotope is shifted, suggesting that this fragment ion still contains the chlorine atom. The largest peak in the daughter spectrum of the protonated ^{37}Cl-molecule occurs at *m/z* 255; it appears as a low intensity ion

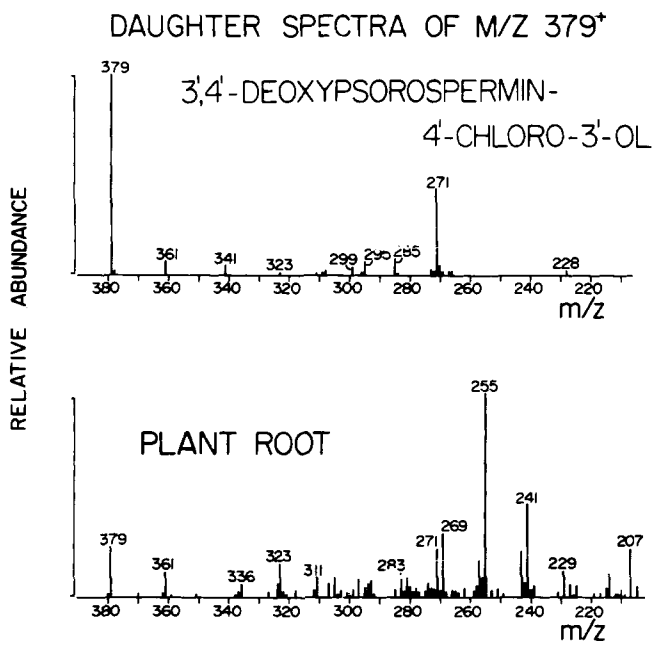


FIGURE 5. Ms/ms daughter spectra of the protonated molecular ion of the ^{37}Cl-isotope of 3′,4′-deoxypsorospermin-4′-chloro-3′-ol (*m/z* 379) and the plant root material at *m/z* 379 recorded at 20 eV collision energy by quadrupole scanning. Comparison of these spectra with the corresponding spectra of Figure 2 indicate that another chlorine-containing compound may be present in the plant root material.

in the spectrum of the ^{35}Cl-isotope and is apparently due to a nonchlorinated component. The agreement between the *m/z* 377 and 379 daughter spectra of the plant root material supports the interpretation that a chlorinated compound or compounds of molecular weight 376.5 is present in the plant. The data of Figure 5, like those of Figure 2, confirm that this compound is not simply the isolated chlorohydrin (**1**), although the presence of **1** is allowed. The characteristic fragment ions, viz. 241, 255, 323, and 361, which occur in the ms/ms spectrum of the authentic compound using the chosen ionization methods and dissociation energies, suggest a similar structure for the component of the plant root material to that of the isolated authentic compound. The probability is low that impurities which interfere with the ^{35}Cl-compound will also interfere in the spectrum of the ^{37}Cl-compound.

Psorospermin was examined in an analogous fashion to the chlorohydrin, viz., by examining both $M^{+\cdot}$ and $(M+H)^+$ ions at low and high collision energies. As expected, these experiments each yield daughter spectra with characteristic fragmentation patterns. The high energy daughter spectrum of authentic protonated psorospermin, shown as the top spectrum in Figure 6, shows the loss of a water molecule result-

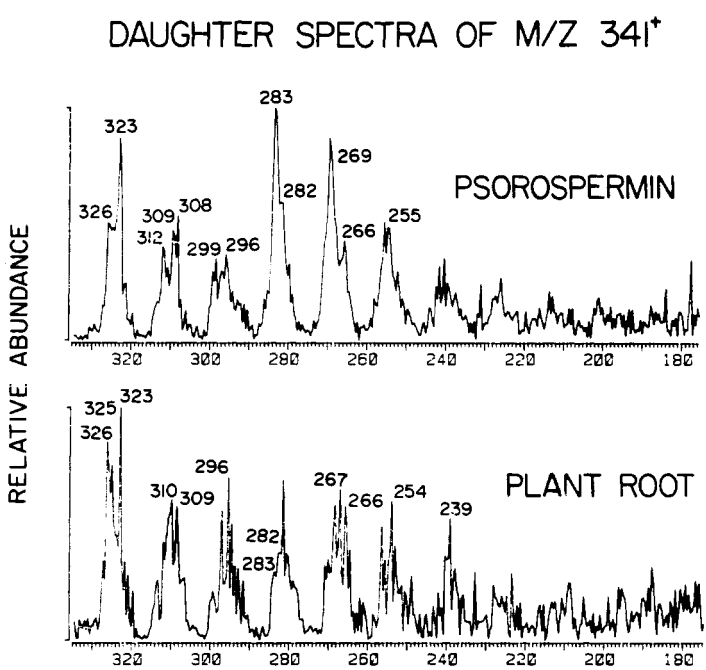
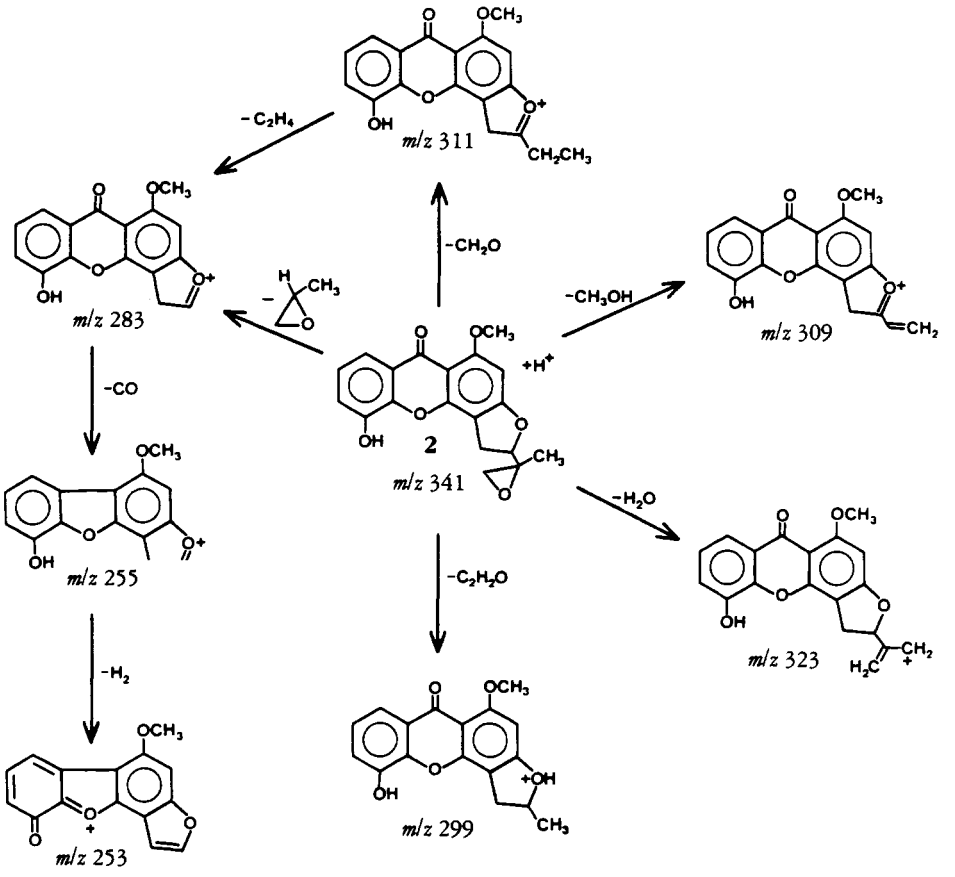


FIGURE 6. Ms/ms daughter spectra of protonated psorospermin (*m/z* 341) and the plant root material at *m/z* 341 recorded at 7000 eV collision energy by ESA scanning.

ing in the ion at *m/z* 323 and the loss of a MeOH molecule yielding an ion at *m/z* 309. These and other fragmentations are rationalized in Scheme 4. Fragmentation of the side chain dominates the spectrum and leads to a variety of stable product ions.

The low energy CID daughter spectrum of protonated psorospermin is shown as a part of Figure 7. The most abundant fragment ion, *m/z* 271, arises from loss of the epoxide side chain, including one carbon atom of the five-membered ring. Fragmentations involving part or all of the epoxide side chain result in the ions *m/z* 311, 299, 283, and 281.

Careful examination of the daughter spectra of *m/z* 341 of the plant root material under both low and high energy conditions suggests that psorospermin is indeed a com-



SCHEME 4. Main fragmentation of protonated psorospermin induced by 7000 eV collisions.

ponent of the plant root. In Figure 7 note the duplication of every peak in the authentic spectrum in that of the root material. Extraneous peaks in the spectrum of the root material are probably due to other components in the matrix. The MIKE spectra of the authentic compound and the plant root material also agree, although these spectra each have a lower signal-to-noise ratio.

The daughter spectra of the molecular ion ($M^{+\cdot}$) under both high and low energy collision conditions give complementary data to that of the protonated molecule. The high energy spectrum shows the loss of ·OH, ·OCH_3, ·CH_3, and the entire side chain, forming ions that are also present in the daughter spectrum of the protonated molecule. The loss of the carbonyl group from the ions at *m/z* 323 and 309 yield ions at *m/z* 295 and 281.

As was the case for the chlorohydrin, the low energy daughter spectrum of the molecular ion of psorospermin shows much more fragmentation than does the protonated molecule. Loss of the methoxy group forms an ion at *m/z* 309 and subsequent loss of the carbonyl group and a hydrogen atom gives rise to ions at *m/z* 281 and 280. The molecular ion readily loses a hydrogen atom forming an ion at *m/z* 339, which loses the carbonyl group to form an ion at *m/z* 311. An ion at *m/z* 241, which was also present in the low energy daughter spectrum of the chlorohydrin, is due to the loss of the entire epoxide side chain. Unfortunately, data on the plant extracts selecting $M^{+\cdot}$ for tandem ms did not add to the evidence available for the presence of psorospermin, and they are therefore not presented.

The greater intensity of the ions comprising the spectra of psorospermin (MW 340)

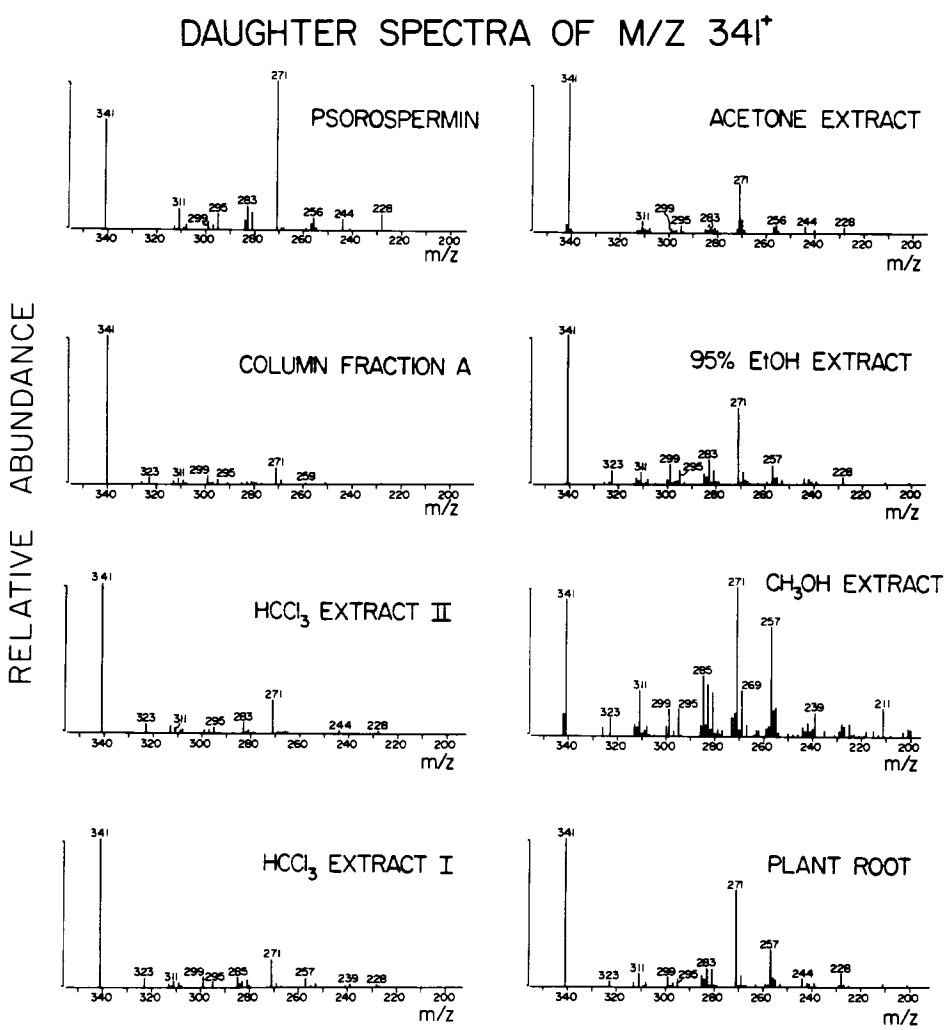


FIGURE 7. Ms/ms daughter spectra of protonated psorospermin (*m/z* 341), samples of the extracted root material after various degrees of purification, and the plant root material at *m/z* 341 recorded at 20 eV collision energy by quadrupole scanning.

in the plant material compared to those arising from psorospermin chlorohydrin (MW 376.5) suggests that psorospermin is present in the plant at a higher concentration than is the chlorohydrin. The most striking contrast in the mass spectral data between the psorospermin and the 3′,4′-deoxypsorospermin-4′-chloro-3′-ol xanthones is the fact that the presence of **2** in the root material is evident in the triple quadrupole data taken on $(M+H)^+$. This result is in marked contrast to that for the chlorohydrin, in spite of the fact that the latter can be extracted from the root in ten times greater amounts. This supports the proposition that the chlorohydrin does not occur in major part per se, in the root material.

Based on previous experience with ms/ms analysis for targeted compounds, the present results lead us to generalize that strongly acidic or basic compounds are easier to identify in complex mixtures than are neutral compounds. This arises because selective ionization simplifies the complexity of the ionic mixture, which is subjected to collision-induced dissociation in tandem ms. In the absence of such selectivity, it is extremely valuable to have available data from more than one method of ionization or to

employ different conditions of dissociation. This increases the probability of obtaining a spectrum that is free of interference.

It is noteworthy that the contribution made to the daughter spectrum of the analyte by other constituents of the mixture was often greater in the extract samples than in the root sample. In previous work on alkaloids (11), better signal-to-noise ratios and matches with authentics invariably were associated with sample clean-up. Contributions from column bleed and incomplete availability of all the compounds in the root for ionization are two reasons that may contribute to the present unusual behavior. In several instances, the plant root material provides a better matrix from which to detect psorospermin than do the purer extracts.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. S.M. Kupchan, D.R. Streelman, and A.T. Sneden, *J. Nat. Prod.*, **43**, 296 (1980).
2. J.M. Cassady, C.J. Chang, A. Habib, D. Ho, A. Amonkar, and S. Masuda, Antineoplastic Agents From Higher Plants: Novel Xantones from *Psorospermum febrifugum*, in: "Natural Products and Drug Development." Ed. by P. Krogsgaard-Larsen, S.B. Christensen, and H. Kofod, Munksgard, Alfred-Benzon Symposium No. 20, 1984, pp. 228-237.
3. F.W. McLafferty, *Science*, **214**, 280 (1981).
4. F.W. Crow, K.B. Tomer, and M.L. Gross, *Mass Spectrom. Rev.*, **2**, 47 (1983).
5. K.E. Singleton, R.G. Cooks, and K.V. Wood, *Anal. Chem.*, **55**, 762 (1983).
6. R.A. Roush and R.G. Cooks, *J. Nat. Prod.*, **47**, 197 (1984).
7. W.C. Brumley, Z. Min, J.E. Matusic, J.A.G. Roach, C.J. Barnes, J.A. Sphon, and T. Fazio, *Anal. Chem.*, **55**, 1405 (1983).
8. R. Endele and M. Senn, *Int. J. Mass Spectrom. Ion Phys.*, **48**, 81 (1983).
9. J.V. Johnson, R.A. Yost, and K.F. Faull, *Anal. Chem.*, **56**, 1655 (1984).
10. P. Rinaldo, L. Chiandetti, F. Zacchello, S. Daolio, and P. Traldi, *Biomed. Mass Spectrom.*, **11**, 643 (1984).
11. R.A. Roush, R.G. Cooks, S.A. Sweetana, and J.L. McLaughlin, *Anal. Chem.*, **57**, 109 (1985).
12. K.E. Singleton, R.G. Cooks, K.V. Wood, K.T. Tse, and L. Stock, *Anal. Chim. Acta*, **174**, 211 (1985).
13. T.L. Kruger, R.W. Kondrat, K.T. Joseph, and R.G. Cooks, *Anal. Biochem.*, **96**, 104 (1979).
14. D. Prome, C. Lacave, J. Roussel, and J.C. Prome, *Biomed. Mass Spectrom.*, **9**, 527 (1982).
15. H.I. Kenttämaa and R.G. Cooks, *Int. J. Mass Spectrom. Ion Processes*, **64**, 79 (1985).
16. J.H. Beynon, R.G. Cooks, J.W. Amy, W.E. Baitinger, and T.Y. Ridley, *Anal. Chem.*, **45**, 1023A (1973).
17. J.R.B. Slayback and M.S. Story, *Ind. Res. Dev.*, 128 (1981).
18. G.W.A. Milne, H.M. Fales, and T. Axenrod, *Anal. Chem.*, **43**, 1815 (1971).
19. W.J. Richter and H. Schwarz, *Angew. Chem. Int. Ed. Engl.*, **17**, 424 (1978).
20. K. Levsen and H. Schwarz, *Mass Spectrom. Rev.*, **2**, 77 (1983).
21. J.D. Ciupek, D. Zakett, R.G. Cooks, and K.V. Wood, *Anal. Chem.*, **54**, 2215 (1982).
22. H.I. Kenttämaa and R.G. Cooks, *J. Am. Chem. Soc.*, **107**, 1881 (1985).
23. S. Verma, J.D. Ciupek, and R.G. Cooks, *Int. J. Mass Spectrom. Ion Processes*, **62**, 219 (1984).
24. S.A. McLuckey and R.G. Cooks, *Int. J. Mass Spectrom. Ion Processes*, **56**, 223 (1984).
25. M.G. Inghram, G.R. Hanson, and R. Stockbauer, *Int. J. Mass Spectrom. Ion Phys.*, **33**, 253 (1980).
26. J. Dannacher, A. Schmelzer, J.P. Stadelmann, and J. Vogt, *Int. J. Mass Spectrom. Ion Phys.*, **31**, 175 (1979).
27. P. Arends, P. Helboe, and J. Moller, *Org. Mass Spectrom.*, **7**, 667 (1973).
28. S. Ghosal, P.V. Sharma, R.K. Chaudhuri, and S.K. Bhattacharya, *J. Pharm. Sci.*, **62**, 926 (1973).
29. S. Ghosal, R.K. Chaudhuri, and A. Nath, *J. Pharm. Sci.*, **62**, 137 (1973).
30. R.K. Chaudhuri and S. Ghosal, *Phytochemistry*, **10**, 2425 (1971).
31. V.V.S. Murti, T.R. Seshadri, and S. Sivakumaran, *Phytochemistry*, **11**, 2089 (1972).

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