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A RAPID SCREENING METHOD FOR ARTEMISININ AND ITS CONGENERS USING MS/MS: SEARCH FOR NEW ANALOGUES IN *ARTEMISIA ANNUA*

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ABSTRACT.—A rapid screening method based on tandem mass spectrometry (ms/ms) is described for artemisinin-related compounds present in complex matrices. These compounds produce abundant ammonium adducts, $[M+NH_4]^+$, using ammonia desorption chemical ionization (dci), and dissociation of the mass-selected adducts yields the protonated molecules, $[M+H]^+$, which subsequently eliminate characteristic neutral molecules (H_2O, CO, HCO_2H, HOAc). Neutral loss ms/ms scans which are selective for different elimination reactions were used in order to screen for groups of related analogues present in a crude hexane extract of *Artemisia annua*. Comparison of ms/ms product spectra of known *Artemisia* compounds with those of the new analogues provided information on the functional groups and the molecular weights of the new compounds present in the plant, and tentative structures are suggested.

Artemisinin (qinghaosu), isolated from *Artemisia annua* L. (Compositae), is an effective antimalarial drug against both chloroquinine-resistant and chloroquinine-sensitive strains of *Plasmodium falciparum*, as well as against cerebral malaria (1–4). It has also been found to be a potential agent for treating skin diseases (5) and as a natural herbicide (6,7). Artemisinin [**1**] has a unique structure among antimalarial agents, lacking the nitrogen-containing heterocyclic ring system found in most. It is a sesquiterpene lactone with an endoperoxide moiety, a functionality rare in natural products. The significant biological activity, novel chemical structure, and low yield from natural sources have prompted efforts directed at its synthesis and at the isolation of related compounds with similar activities (1). The discovery of these new compounds has provided useful information on biosynthetic pathways and structure-activity relationships and has been invaluable in developing more potent antimalarial drugs. For example, a synthetic analogue of artemisinin, containing the endoperoxide moiety but lacking the 1′,2′,4′-trioxane ring system, failed to show significant antimalarial activity (8). This is evidence (9) that the steric environment of the trioxane ring system is as vital as the peroxide group for expressing antimalarial activity. In other studies, semi-synthetic derivatives of artemisinin [amines (10), ethers (11), esters (12), and carboxylic acids (13)] have shown higher activity than the parent compound. Although related compounds present in *A. annua* may not have direct pharmacological activity, they might still have medicinal importance. For example, artemisinic acid, a relatively abundant constituent of *A. annua*, has been used as a precursor (14,15) in the synthesis of the relatively minor, but far more bioactive constituent of the same plant, artemisinin. Artemisinic acid is also known (16) to be the biogenetic precursor of artemisinin. Although several total syntheses of artemisinin and its analogues (1,17,18) have been reported, *A. annua* appears to be the sole economic source of this important class of compounds. This justifies searches for additional artemisinin-related compounds in *A. annua*.

The ability to detect artemisinin and its known analogues in plant extracts is as critical as the isolation of new compounds. This task is especially difficult when these compounds are present at trace levels in a complex matrix. Although methods for analyzing these compounds presently exist (19–24), they require pure samples and/or extensive prior separation. Rapid methods of detection at trace levels that are applicable

to mixtures are still needed. The thermal lability and acid sensitivity of artemisinin and its analogues can cause extra difficulty in detection because products of thermal or acid degradation can interfere and be falsely identified as new analogues in the plant matrix or as drug metabolites in biological fluids. Tandem mass spectrometry (ms/ms) (25,26) is a powerful technique for detecting a target compound in complex matrices, including plant extracts (27–29) and biological fluids (30). The strength of this technique is due to the selectivity, high sensitivity, and fast screening capabilities compared to many other separation and identification techniques. Soft ionization methods such as chemical ionization (ci) (31) and desorption ionization (32) often facilitate selective ionization of the desired analyte and, by depositing less internal energy into the molecule upon ionization, decrease the extent of fragmentation and increase the abundance of the molecular ion of interest. Mass-selection of this ion using the first mass analyzer of a tandem mass spectrometer allows one to obtain an ms/ms product spectrum using the second analyzer after inducing fragmentation in the region between the analyzers. The aim of this study is to develop an ms/ms method of analyzing artemisinin-related compounds already known to be present in *A. annua* and to apply this method to screen for as yet unknown naturally occurring analogues of artemisinin which, after full structural characterization, could prove to be even more potent as antimalarial agents.

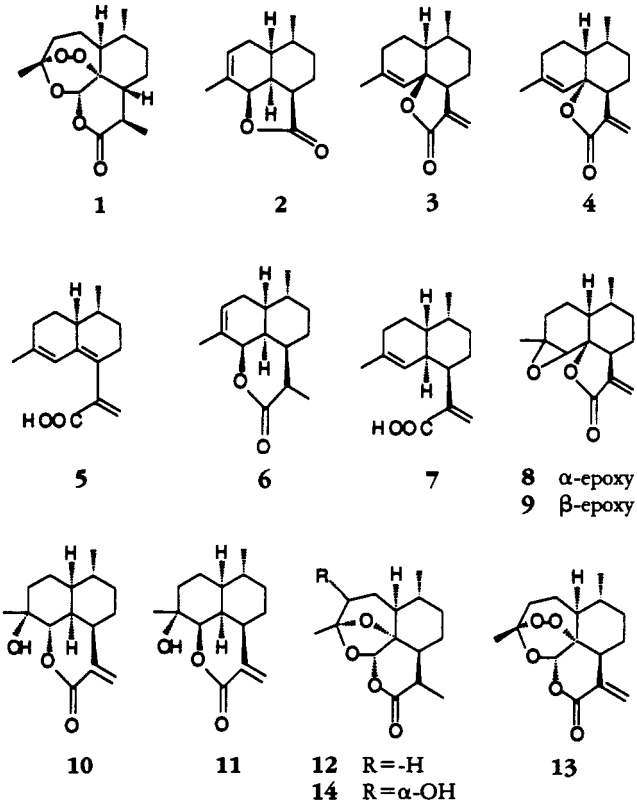
EXPERIMENTAL

Experiments were performed using a Finnigan TSQ 700 mass spectrometer. A few μl of the sample solution (hexane extract of the leaves or authentic artemisinin solution in hexane) was transferred to the loop of rhenium wire filament of the direct insertion probe and dried in air before introduction into the ion source. The amount of sample loaded onto the probe tip was approximately 0.5 μg. Electron energy was 70 eV. The filament was heated by passing a current of 0.2 mA through it, which caused heating from ambient temperature to 200° at a rate of 100°/sec. Positive ion desorption chemical ionization (dci) used either NH_3 (0.5 torr) or isobutane (0.5 torr) as the reagent gas. Isobutane produced $[M+H]^+$, whereas NH_3 reagent gas also produced $[M+NH_4]^+$ ions, where M indicates the analyte. The source temperature was maintained at 150° for both isobutane dci and NH_3 dci. Argon was used as collision gas for ms/ms experiments at a pressure of 2.0×10^{-3} torr, measured using an uncorrected ion gauge connected to the collision cell. Crude plant leaf extract (in hexane) of *A. annua* and the authentic artemisinin were obtained from Prof. John M. Cassadey, then of the Department of Medicinal Chemistry and Pharmacognosy, Purdue University.

RESULTS AND DISCUSSION

Apart from artemisinin [**1**], thirteen closely related terpenoid lactones (or carboxylic acids) have been identified in *A. annua*: artemisin A [**2**], dexoyisoartemisinin B [**3**], deoxyisoartemisinin C [**4**], dehydroartemisinic acid [**5**], compound **6** (unnamed), 6,7-artemisinic acid [**7**], artemisinin B [**8**], artemisinin C [**9**], qinghaosu VI [**10**], qinghaosu V [**11**], deoxyartemisinin [**12**], artemisitene [**13**], and qinghaosu IV [**14**].

Artemisinin and related compounds are known (33–35) to produce significant fragments even under soft ionization conditions (ci, fab, and thermospray). Dci was chosen for the study of artemisinin because it has proven effective in generating molecular ions of a variety of other compounds without causing much fragmentation (36). Because it is a rapid heating technique, thermal degradation is also minimized. This is a particularly useful feature of dci, because artemisinin-related compounds are thermally labile. The effect of experimental conditions (choice of reagent gas, the use of dci vs. conventional ci) on fragmentation can be illustrated by comparing the isobutane ci with the NH_3 dcims of an authentic sample of artemisinin. The isobutane cims of authentic artemisinin shows significant fragmentation: *m/z* (%) 565(17) $[2M+H]^+$, 283(22) $[M+H]^+$, 265(48), 247(22), 237(30), 223(3), 219(25), 209(100), 191(5). NH_3 dci shows only the $[M+NH_4]^+$ adduct ion, *m/z* 300, under experimental conditions which are otherwise the same. An interesting feature of the isobutane ci spectrum is the



peak corresponding to the proton-bound dimer, *m/z* 565. The proton-bound dimers of this class of compounds are also known to occur under fab conditions (34).

The use of NH_3 dci is fundamental to the success of this study. By choosing an appropriate reagent gas, the amount of internal energy deposited in the analyte upon ionization can be minimized, maximizing the formation of protonated molecules $[M+H]^+$ and/or ion-molecule adducts $[M+N]^+$, where N indicates the neutral reagent. Minimal fragmentation of $[M+N]^+$ or $[M+H]^+$ is important for complex mixture analysis for two reasons. First, by reducing the extent of fragmentation, chemical interference is limited to molecular ions of isomers and isobars present in the mixture because isobaric interferences due to fragment ions from higher mass parent ions are eliminated. Second, by increasing the fraction of the ion current carried by $[M+H]^+$ or $[M+N]^+$, the parent ion in ms/ms experiments, lower limits of detection are achieved.

Figure 1 compares the higher mass regions of the dcims recorded for the crude hexane extract of *A. annua* using isobutane (Figure 1a) and NH_3 (Figure 1b) as reagent gases. Several interesting observations on the spectra can be made. First the S/N ratio for the artemisinin-ammonium adduct, *m/z* 399, in the NH_3 spectrum is higher than that for the corresponding protonated molecule, *m/z* 283, in the isobutane spectrum. This is partly due to the decrease in fragmentation as noted earlier. Another reason might be the propensity of these compounds towards NH_3 adduct formation. Some reagent gases are known to form specific ion/molecule adducts, and these have been used to distinguish particular classes of natural products (37). Arteether (a semi-synthetic artemisinin derivative) and its metabolites are also known to produce abundant NH_3 adducts under thermospray ionization conditions (35). Another observation is that those artemisinin-related compounds that include lactone and acid functionalities form abundant NH_3

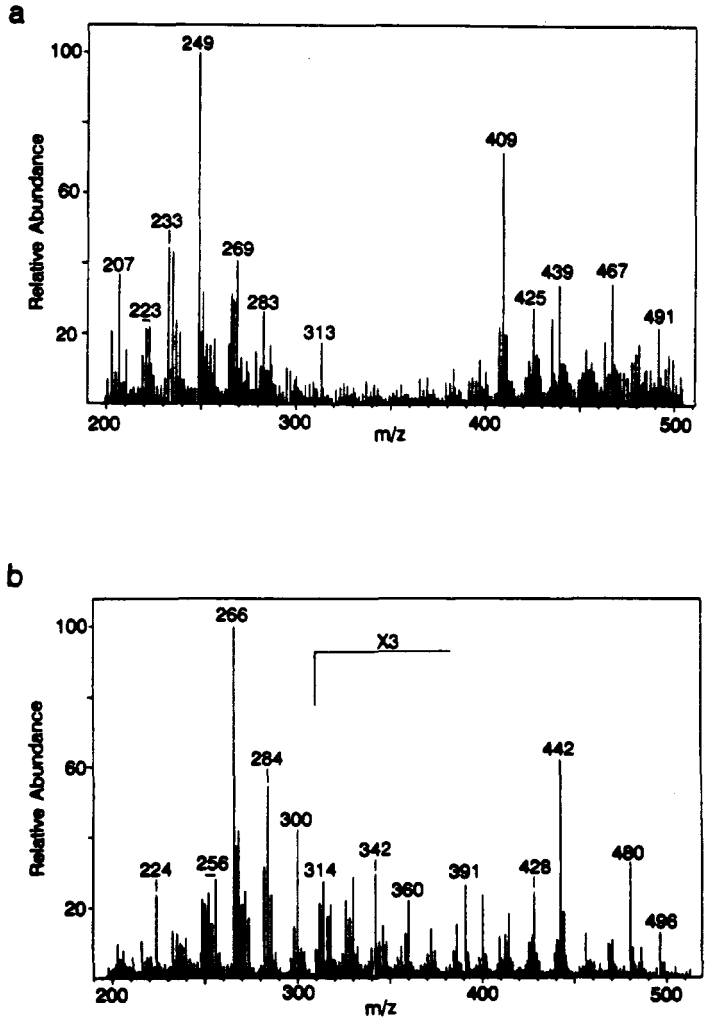


FIGURE 1. Comparison of dci mass spectra of the leaf extract of the plant *Artemisia annua*, using two different reagent gases: (a) isobutane and (b) NH_3.

adducts. For example artemisinin [**1**], the pair of isomers **8,9** (mol wt 248), and **12** (mol wt 266) can be associated with abundant peaks due to NH_3 adducts in the NH_3 dci spectra at *m/z* 300, m/z 266, and *m/z* 284, respectively. One might expect that the other intense NH_3 adduct peaks, $[M+NH_4]^+$, are also due to compounds with these functional groups.

In order to screen for analogues of a certain target molecule, two steps must be taken (38). The first deals with the analysis of the ms/ms product spectrum of the target compound. This analysis yields the ability to recognize the characteristic fragments or neutral losses arising from the ionized form of this molecule. Then in the second step, this information is utilized together with additional ms/ms scans in order to identify the unknowns. The hypothesis on which such a procedure is based is that the ionic form of the unknowns will behave similarly to the ionized form of the authentic compound, and hence their spectra will share common characteristics (specific fragment ions and common neutral losses). Ms/ms alone is insufficient to confirm the structures. However,

combination of several ms/ms scans can provide useful information, particularly regarding the functional groups present.

Consider the ms/ms product spectrum of the ammonium adduct of artemisinin, *m/z* 300 (Figure 2). The adduct yields an abundant fragment ion *m/z* 283 which corresponds to protonated artemisinin itself. The other fragments observed are similar in *m/z* values to the ions observed in the isobutane ms, suggesting that the fragments which occur under collision activated dissociation (CAD) originate from the protonated ion after the initial loss of NH_3. The ions at *m/z* 265, 247, and 229 are due to the sequential loss of H_2O molecules, in addition to NH_3 , from the adduct. The loss of NH_3+H_2O+CO (or NH_3+HCO_2H; mass 63) yielding the ion at *m/z* 237, and the loss of NH_3+2H_2O+CO (or $NH_3+H_2O+HCO_2H$; mass 81) yielding *m/z* 219 are also observed in the spectrum. The most characteristic fragmentation occurs with the loss of a neutral molecule(s) of mass 77 producing the ion, *m/z* 223. The characteristic neutral loss of 60 from artemisinin under both ei and ci conditions is known (33,34) to be due to HOAc. The loss of HOAc from the NH_3 adduct of artemisinin is rationalized by Scheme 1. Further evidence for the formation of intermediate **a** comes from the study of terpenoid esters under isobutane ci conditions. The acetate esters of the terpenoids are known to produce abundant peaks corresponding to $[MH-HOAc]^+$ in their ci spectra (39).

The ms/ms product spectra of artemisinin and a number of known analogues, recorded by performing experiments on the plant matrix, are summarized in Table 1.

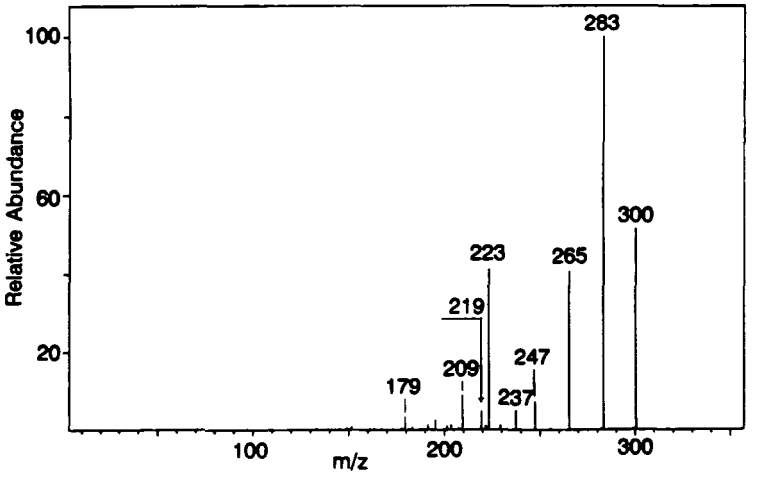
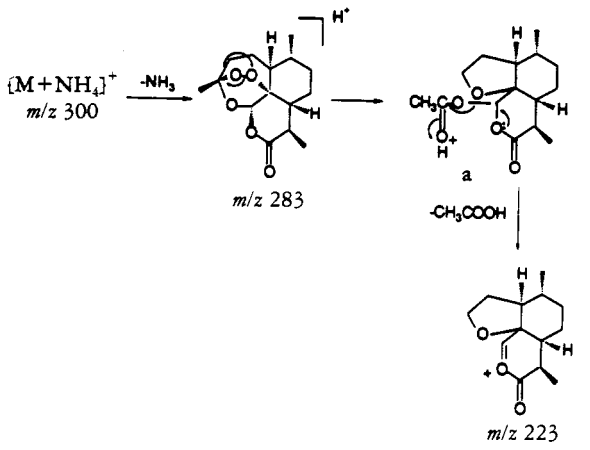


FIGURE 2. The ms/ms product spectrum of *m/z* 300, the NH_3 adduct of artemisinin (authentic) under NH_3 dci conditions.

The presence of artemisinin in the plant leaf extract is confirmed by comparing the product spectrum of the ion, *m/z* 300 (Table 1), and that obtained from the authentic sample (Figure 2). The product spectra of known analogues also share common features with that of artemisinin. The most common feature is that the NH_3 adducts of all the known analogues first lose NH_3, yielding $[MH]^+$, and subsequently eliminate characteristic neutrals (H_2O, HCO_2H, CO, etc.). Importantly, the formation of $[NH_4]^+$ is not evident in any of the spectra under CAD conditions, indicating that the proton affinities (PA) of these compounds are higher than that of NH_3 (858 kJ/mol). This conclusion follows from the fact that the PA's of the molecules comprising the proton-bound dimer, $NH_3.H^+$. M, are reflected in the ion abundance ratio, $[MH]^+/[NH_4]^+$, in the product spectrum (40). In particular cases, it has been found that when the PA difference between



SCHEME 1

the consistent bases of the dimer was higher than 2 kJ/mol, only one protonated ion is observed upon CAD of the proton bound dimer (41). As the PA of M becomes increasingly greater than that of NH_3, the $MH^+...NH_3$ bond becomes weaker, favoring the rapid decomposition of the complex. In these cases, only $[MH]^+$ is observed in the ion source, under typical NH_3 Ci conditions (42). The fact that mainly $[M+NH_4]^+$ and very little $[MH]^+$ are observed in the NH_3 dcims suggests that the PA of these compounds is higher but not much higher than that of NH_3 (858 kJ/mol). Although isolated carboxylic acids, esters, and lactones have much lower PA's (795–815 kJ/mol) than NH_3 (858 kJ/mol) the extra stabilization provided by other oxygen functional groups can raise the PA of carboxylic acids and lactones closer to the PA of NH_3 (42). These arguments, and the experimental observations, suggest that artemisinin analogues are expected to have a narrow range of PA values, probably little higher than NH_3. The fact that new analogues discussed below also have PA's similar to those of known analogues can be used to provide a method to search for new analogues. This is accomplished by performing ms/ms scans for neutral loss 17 daltons, as described below.

A neutral loss (NL) scan is done by scanning both the first and third quadrupoles in the triple quadrupole simultaneously with a constant offset mass (43). In order to yield high abundances in the NL 17 spectrum, the analyte not only has to form an abundant ammonium adduct, it also should readily give rise to the protonated ion upon CAD. As

TABLE 1. The NH_3 dci/ms/ms Product Spectra of NH_3 Adducts $[M+18]^+$ of the Known Artemisinin Analogues Obtained by Mass Selecting Corresponding Parent Ions Generated from the Plant Extract.

Mol wt (M)	Compound	*m/z* (% relative abundance)
282	**1,14**	300 (51), 283 (100), 265 (40), 247 (7) 237 (5), 223 (41), 219 (5), 209 (9), 195 (3), 179 (3)
206	**2**	224 (62), 207 (100), 189 (3), 161 (8)
232	**3,4,5**	250 (71), 233 (100), 215 (6), 205 (2)
234	**6,7**	252 (44), 235 (100), 217 (8), 189 (2), 179 (2), 161 (2)
248	**8,9**	266 (100, 249 (94), 231 (36), 213 (5), 203 (5), 189 (20), 185 (13), 177 (5)
250	**10,11**	268 (52), 251 (100), 233 (58), 215 (2), 205 (5), 191 (2), 187 (3)
266	**12**	284 (90), 267 (100), 249 (19), 231 (8), 221 (4), 209 (2), 207 (3), 203 (2)
280	**13**	298 (72), 281 (100), 263 (43), 245 (11), 235 (6), 221 (5), 217 (2), 207 (2)

expected, the known analogues give abundant signals in the NL 17 spectrum (Figure 3). Note that this spectrum is less complex than the mass spectrum of the leaf extract (Figure 1), because the compounds that do not satisfy the two conditions mentioned above are eliminated. The additional abundant ions appearing in the spectrum (Figure 3) are candidates for previously unknown analogues of artemisinin. Discrimination against

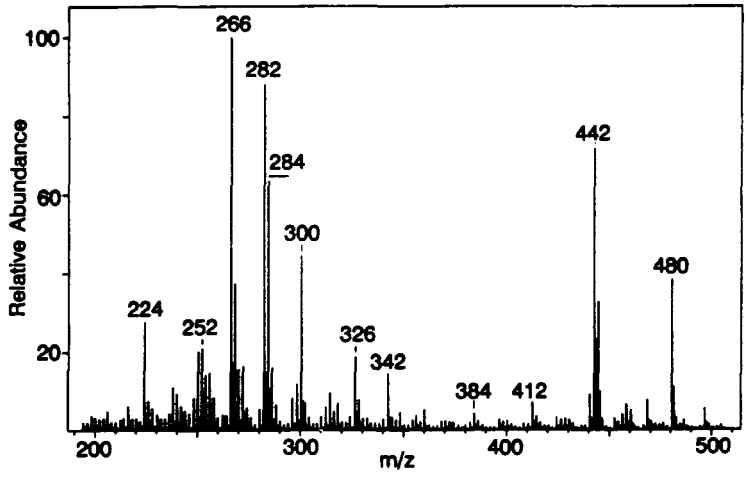


FIGURE 3. The ms/ms neutral loss (offset 17; NL 17) spectrum of the leaf extract under NH_3 dci conditions.

false positives in the NL 17 screen can be achieved by performing other neutral loss scans. As discussed earlier, the NH_3 adducts of all the known analogues lose NH_3, followed by loss of neutral molecules such as H_2O, CO, HCO_2H, etc. upon CAD. Scans for these combined neutral losses were used to obtain the spectra shown in Figure 4. Since the artemisinin analogues have similar functionalities, they are expected to fragment similarly and to give abundant peaks in most of the NL spectra shown (Figures 4 and 5). Therefore, the next step in identifying possible new artemisinin analogues involves choosing the adducts that produce abundant signals in most of the NL scans. The ms/ms product spectra of those compounds are then obtained and compared with ms/ms product spectra of the knowns. By means of this procedure, the functional groups present in the unknowns can be identified. In a few cases, possible structures of the new analogues can be proposed. Note that the comparison of the product ms/ms of unknowns with that of knowns is complicated in some cases because the ms/ms product spectra of the known compounds obtained from the leaf extract represent a mixture of isomers. Naturally if the authentic samples of all the knowns were available, comparison would be much easier. However, the objective of this work is to provide information on the molecular weights and possible functionalities of the new analogues.

As an example of the value of this approach in providing useful insights into the functionalities present in the unknown structures, consider neutral loss 77 (Figure 5b). As already noted, this spectrum represents the adducts which lose NH_3 and also subsequently eliminate a neutral of 60 daltons, presumably HOAc (Scheme 1). Notice that only a few abundant ions, including *m/z* 266, 284, and 300 (the ammonium adduct of artemisinin itself), show this characteristic behavior. The loss 77 from the ion *m/z* 266 is ascribed to the ammonium adducts of analogues **8** and **9**. These compounds do not have the intact MeC(O)O functionality found in artemisinin itself (Scheme 1), so direct loss of HOAc from protonated **8** and **9** is not feasible without a complex rearrangement

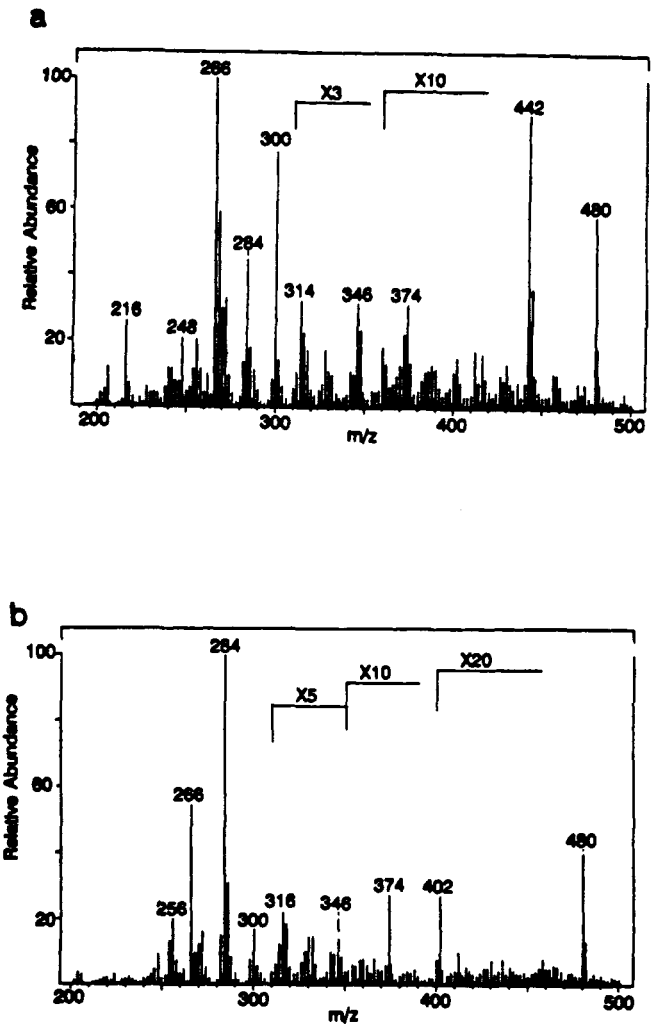


FIGURE 4. The ms/ms neutral loss (NL) spectra of the leaf extract under NH_3 *dci conditions*: (a) NL 35 (b) NL 53.

prior to the fragmentation. However, the combination of three neutral losses (NH_3, $CH_2{=}C{=}O$, and H_2O) can also give rise to the mass, 77, of which $CH_2{=}C{=}O$ is expected to originate from the epoxide group of the compounds. The other neutral molecule in the combination, H_2O, can originate from the lactone group. These observations suggest that the unknown adducts which undergo loss of 77 daltons, ions *m/z* 442 and 486, should also have Me-CO functional groups in their structures. Note that ion *m/z* 409, which is the result of losing 77 daltons, is the most abundant peak in the product spectrum of ion *m/z* 486 indicating the strong possibility of the presence of the MeC(O)O functional groups in this compound. In addition to NL of 77, the ms/ms product spectra of these analogues (Table 2) also show other common neutral losses, NH_3, H_2O, CO, etc., suggesting the presence of -OH, $-CO_2H$ and/or -COOR functionalities.

Another characteristic neutral loss, 81, is shown in Figure 5c. Very few adducts, including the knowns present in the previous spectrum (Figure 5b), are apparent in the spectrum. This loss, 81, is likely due to combinations of neutral molecules, either $NH_3{+}H_2O{+}HCO_2H$ or $NH_3{+}2H_2O{+}CO$. The product spectra of the NH_3 adducts

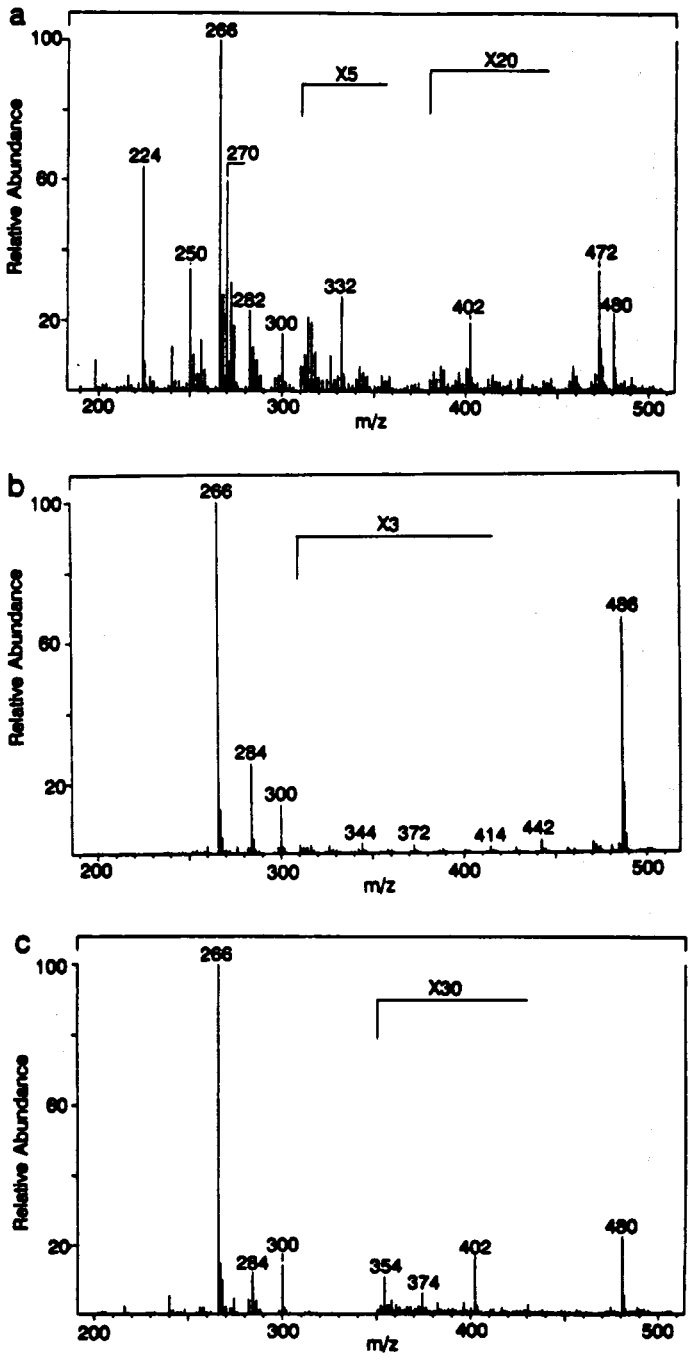


FIGURE 5. The ms/ms neutral loss (NL) spectra of the leaf extract recorded by NH_3 dci: (a) NL 63, (b) NL 77, and (c) NL 81.

of unknowns, *m/z* 402 and 480 (Table 2), also show other initial common losses (H_2O and CO).

An important feature in most of the neutral loss spectra (Figures 3–5) is that the abundant peaks are accompanied by quite intense $[M+2]^+$ and $[M-2]^+$ peaks. It is

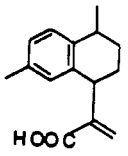
TABLE 2. The NH_3 dci/ms/ms Product Spectra of NH_3 Adducts $[M+18]^+$ of the Possible Artemisinin Analogues and Their Molecular Weights, Obtained by Mass Selecting Corresponding Parent Ions Generated from the Plant Extract.

Mol wt	Compound	*m/z* (% relative Abundance)
230	**15**	248 (100), 231 (43), 213 (36), 203 (2), 195 (4)
236	**16**	254 (100), 237 (100), 219 (30), 209 (3), 201 (7), 191 (2)
252	**17**	270 (65), 253 (100), 235 (77), 217 (8), 207 (16), 193 (2), 189 (2)
268	[a]	286 (65), 269 (100), 251 (58), 233 (31), 223 (8), 215 (2), 209 (3), 205 (6), 191 (2), 187 (2)
284	[a]	302 (83), 285 (100), 267 (88), 249 (14), 239 (5), 231 (4), 225 (6), 221 (3), 207 (3)
292	[a]	310 (91), 293 (100), 275 (23), 265 (4), 257 (3), 247 (6), 233 (7), 231 (5), 205 (2)
294	[a]	312 (56), 295 (100), 277 (30), 249 (6), 249 (5), 235 (3), 233 (3), 231 (3)
296	[a]	314 (54), 297 (100), 279 (58), 261 (5), 251 (8), 233 (4), 183 (3)
308	[a]	326 (25), 309 (100), 291 (10), 273 (2), 263 (3), 249 (2), 231 (2)
310	[a]	328 (99), 311 (100), 293 (77), 275 (11), 265 (7), 249 (20), 233 (5), 231 (11)
312	[a]	330 (75), 313 (100), 295 (71), 277 (10), 267 (6), 253 (4), 251 (9), 249 (5), 239 (7), 233 (3)
384	[a]	402 (100), 385 (41); 367 (22); 349 (6); 339 (4), 323 (3), 321 (8)
424	[a]	442 (23), 425 (100), 407 (8), 365 (2), 299 (2), 245 (4), 231 (3), 177 (2), 163 (4)
426	[a]	444 (19), 427 (100), 409 (5), 233 (2), 219 (5), 205 (4)
462	[a]	480 (7), 463 (100), 445 (6), 207 (2), 177 (2)
468	[a]	486 (21), 469 (52), 451 (4), 423 (3), 409 (100), 249 (5), 231 (5), 221 (3), 219 (9), 203 (6), 191 (6), 78 (6)

[a]Structures are not suggested for these compounds.

evident that many of the known analogues shown consist of the hydrogenated and dehydrogenated analogues of other compounds. Examples of such pairs are **5**,**7** (mol wt 232,234), **13**,**1** (mol wt 280,282) and **9**,**10** (mol wt 248,250). Thus, one might expect that the other abundant peaks in the dci mass spectra, especially $[M+2]^+$ and $[M-2]^+$ peaks, are related to the corresponding known analogues. Consider the ms/ms product spectra of unknown adducts, *m/z* 248 (**15**) and *m/z* 254 (**16**) (mol wt 230 and 236; Table 2). The fact that **15** differs from the known analogues **3**,**4**,**5** (mol wt 232) by two mass units and **16** differs from **6** and **7** (mol wt 234) by two mass units, suggests that **15** may be a dehydrogenated analogue of **3**,**4**, or **5**. Similarly, **16** might be a hydrogenated analogue of **6** or **7**. Both **15** and **16** also show the initial loss of H_2O and CO from the protonated molecules, indicating the presence of functionalities such as -C=O, -OH and -O-. Clearly, there can be several isomeric analogues that fit the above mentioned characteristics. Possible structures for the unknown analogues **15** and **16** are shown. The fact that the product ms/ms of the unknown adduct **17** (Table 2) is very similar to that of the known analogues **10**,**11**, with fragments differing by 2 mass units from each other, suggests that **17** is a hydrogenated analogue of **10**,**11**. A possible structure for **17** is given. Similar arguments suggest that the unknown ion *m/z* 302, is a hydrogenated analogue of **12**, but its structure cannot be established by ms/ms experiments alone.

The combination of several NL experiments revealed several unknowns which have similar structural features to the known analogues of artemisinin. Ms/ms product spectra of these new analogues having molecular weights of 284, 292, 294, 296, 308, 310, and 312 are shown in Table 2. The ms/ms product spectra of the analogues, mol wt 284, 294, and 312 closely resemble the fragmentation pattern of artemisinin [**1**]. Note the initial sequential loss of H_2O molecules, in addition to the loss of NH_3, and the characteristic



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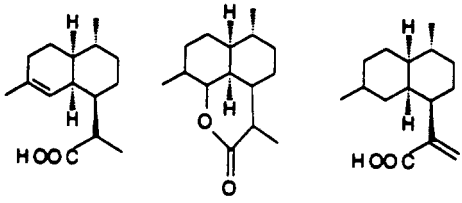

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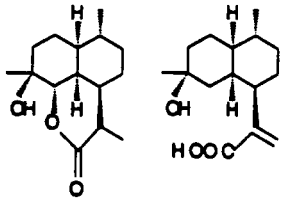

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loss of 77 and 81 which are seen in all the ms/ms product spectra (Table 2). Due to the complex nature of the fragmentation patterns, full structures cannot be predicted. Although the ms/ms product spectra of unknowns with mol wt 292 and 308 do not show the loss of 81, the characteristic loss of 77 as well as other common neutral losses (H_2O, CO etc.) is observed. The unknowns with mol wt 296 and 310 do not closely resemble artemisinin, but the initial loss of H_2O and CO and the fact that they all produce intense NH_3 adducts in the NH_3 dcims of the plant extract suggests they have structures similar to those of the known analogues present in the plant.

In conclusion, the rapid screening method for artemisinin-related compounds developed in this study will be useful in various applications including (i) pharmacokinetic studies of these potential drugs, (ii) characterization of drug metabolites, and especially (iii) screening and detection of natural products with terpenoid lactones and ester functionalities. Although the complete elucidation of the structures of new analogues present in *A. annua* is not possible, molecular weight and functional group information has been obtained by ms/ms and structures of several analogues are suggested (e.g., **15** and **16**). The structure elucidation of the suggested new analogues should be possible using the present data in combination with other analytical techniques.

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LITERATURE CITED

1. S.S. Zamand and R.P. Sharma, *Heterocycles,* **32**, 1593 (1991).
2. X.-D. Luo and C.-C. Shen, *Med. Res. Rev.*, **7**, 29 (1987).
3. D.L. Klayman, *Science,* **228**, 1049 (1985).
4. J.M. Liu, N.Y. Ni, J.F. Fan, Y.Y. Tu, Z.H. Wu, Y.L. Qu, and M.S. Chou, *Acta Chim. Sin.*, **37**, 129 (1979).
5. C.R.Thornfeldt, Can. Patent CA 2003177 (1991), *Chem. Abstr.*, **116**, 34598 (1992).
6. S.O. Duke, K.C. Vaughn, E.M. Croom, and H.N. Elsohly, *Weed Sci.*, **35**, 499 (1987).
7. P.K. Chen, G.R. Leather, and D.L. Klayman, *Plant Physiol.*, **83S**, Abstract 406 (1987).
8. Y. Imakura, T. Yokoi, T. Yamagishi, J. Koyama, H. Hu, D.R. McPhail, and K.H. Lee, *J. Chem. Soc., Chem. Commun.*, 372 (1988).
9. Y. Imakura, K. Hachiya, T. Ikemoto, S. Yamashita, M. Kihara, S. Kobayashi, T. Shingu, W.K. Milhous, and K.H. Lee, *Heterocycles,* **31**, 1011 (1990).
10. A.J. Lin, L. Li, D.L. Klayman, C.F. George, and J.L. Flippen-Anderson, *J. Med. Chem.*. **33**, 2610 (1990).
11. H. Gu, B. Lu, and Z. Qu, *Acta Pharmacol. Sin.*, **1**, 48 (1980).
12. X. Liu, CN Patent 85100781, 20 Aug 1986; *Chem. Abstr.*, **107**, 78111 (1987).
13. A.J. Lin, M. Lee, and D.L. Klayman, *J. Med. Chem.*, **32**, 1249 (1989).
14. R.J. Roth and N. Action, *J. Nat. Prod.*, **52**, 1183 (1989).
15. R.K. Haynes and S.C. Vonwiller, *J. Chem. Soc., Chem. Commun.*, 451 (1990).
16. A. Akhila and S.P. Popli, *Phytochemistry,* **26** 1927 (1987).
17. M.A.Avery, W.K.M. Chong, and C. Jennings-White, *J. Am. Chem. Soc.*, **114**, 974 (1992).
18. T. Ravindranathan, M.A. Kumar, R.B. Menon, and S.V. Hiremath. *Tetahedron Lett.*. **31**, 75 (1990).
19. H.M. Luo, P.-P. Chao, C.-C. Yu, C. Tai, and C.-W. Liu, *Yao Hseueh T'ung Pao.* **15**, 8 (1980).
20. R. Zhang, S. Xu, Y. Li, *Acta Pharm. Sin.*, **16**, 460 (1981).
21. R. Liersch, H. Soicke, C. Stehr, and H.-U. Tullner, *Planta Med.*. **5**, 387 (1986).
22. N. Action, D.L. Klayman, and I.J. Rollman, *Planta Med.*. **5**, 445 (1985).
23. D.J. Charles, J.E. Simon, K.V. Wood, and P. Heinstein, *J. Nat. Prod.*. **53**, 157 (1990).
24. A.T. Sipahimalani, D.P. Fulzele, and M.R. Heble, *J. Chromatogr.*. **538**, 452 (1991).
25. K.L. Busch and R.G. Cooks, in: "Tandem Mass Spectrometry." Ed. by F.W. McLafferty, John Wiley and Sons, New York, 1983, Chapter 2.
26. K.L. Busch, G.L .Glish, and S.A. McLuckey, "Mass Spectrometry/Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry," VCH Publishers, New York, 1988.
27. R.G. Cooks, and R.A. Roush, *Chim. Ind. (Milan).* **66**, 539 (1984).
28. R.A. Roush and R.G. Cooks, *J. Nat. Prod.*. **47**, 197 (1984).
29. R.A. Roush, R.G. Cooks, S.A. Sweetana, and J.L. McLaughlin, *Anal. Chem.*. **57**, 109 (1985).
30. W.J. Richter, W. Blum, U.P. Schlunegger, and M. Senn, in: "Tandem Mass Spectrometry." Ed. by F.W. McLafferty, John Wiley and Sons, New York, 1983.
31. A.G. Harrison, "Chemical Ionization Mass Spectrometry," CRS Press, Boca Raton, Florida, 1983.
32. S.J. Pachuta and R.G. Cooks, *Chem. Rev.*. **87**, 647 (1987).
33. K.P. Madhusudanan, R.A. Vishwakarma, S. Balachandran, and S.P. Popli, *Indian J. Chem.. Sect. B.* **28B**, 751 (1987).
34. H.M. Fales, E.A. Sokoloski, L.K. Pannel, Q.L. Pu, D.L. Klayman, A.J. Lin, A. Brossi, and J.A. Kelley, *Anal. Chem.*. **62**, 2494 (1990).
35. J.K. Baker, R.H. Yarber, C.D. Hufford, I.-S. Lee, H.N. ElSohly, and J.D. McChesney, *Biomed. Environ. Mass Spectrom.*. **18**, 337 (1988).
36. A. Guarini, G. Guglielmetti, N. Andriollo, and M. Vincenti, *Anal. Chem.*. **64**, 204 (1992).
37. R.R. Pachuta, H.I. Kenttamaa, R.G. Cooks, T.M. Zennie, C. Ping, C.-j. Chang, and J.M. Cassady, *Org. Mass Spectrom.*. **23**, 10 (1988).
38. R.J. Perchalski, R.A. Yost, and B.H. Wilder, *Anal. Chem.*. **54**, 1466 (1982).
39. G. Lange and W. Schultze, *Org. Mass Spectrom.*. **27**, 481 (1992).
40. S.A. McLuckey, R.G. Cooks, and J.E. Fulford, *Int. J. Mass Spectrom. Ion Processes.* **52**, 165 (1983).
41. B.D. Nourse and R.G. Cooks, *Int. J. Mass Spectrom. Ion Processes.* **106**, 249 (1991).
42. J.B. Westmore and M.M. Alauddin, *Mass Spectrom. Rev.*. **5**, 381 (1986).
43. J.C. Schwartz, A.P. Wade, C.G. Enke, and R.G. Cooks, *Anal. Chem.*. **62**, 1809 (1990).

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