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PROFILING TAXANES IN *TAXUS* EXTRACTS USING LC/MS AND LC/MS/MS TECHNIQUES

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ABSTRACT.—Analytical methodology developed for the trace analysis of natural products in crude extracts was utilized for the rapid and systematic structure elucidation of taxanes in *Taxus* extracts and process intermediates. This method integrates analytical hplc, uv detection, uv spectroscopy, full-scan ionspray mass spectrometry and tandem mass spectrometry on-line. The identification of structure of a taxane is based on comparing the mass spectrometric characteristics of the taxane with the paclitaxel substructural "template." Analytical data for taxanes in preparations from *Taxus brevifolia* and *Taxus baccata* were observed, including chromatographic characteristics using a standard hplc system, molecular weight, and collision induced dissociation (cid) tandem mass spectrometry (ms/ms) product ion spectra. The data obtained for 18 taxanes from natural sources using this method provided a taxane profile database useful for the rapid identification of taxanes in mixtures and samples of limited quantity.

Paclitaxel (known also in the literature as taxol) is a member of the *Taxus* alkaloid family of natural products and provides a novel treatment for refractory ovarian tumors (1). The mechanism of action of paclitaxel involves the promotion of tubulin monomer assembly into stable dysfunctional microtubules, thus interfering with mitosis (2,3). Many taxanes have been identified over the past 30 years (1, 4–13), and structure-activity studies have shown that paclitaxel's unique structure (Figure 1) is highly favorable for its promotion of tubulin polymerization and cytotoxicity (3).

Previous reports have utilized tandem mass spectrometry (ms/ms) (14,15) and lc/ms/ms for the study of drugs and natural products, such as flavones (16), artemisinin congeners (17), and taxanes (18–23). Schemes for the ms/ms dissociation of paclitaxel

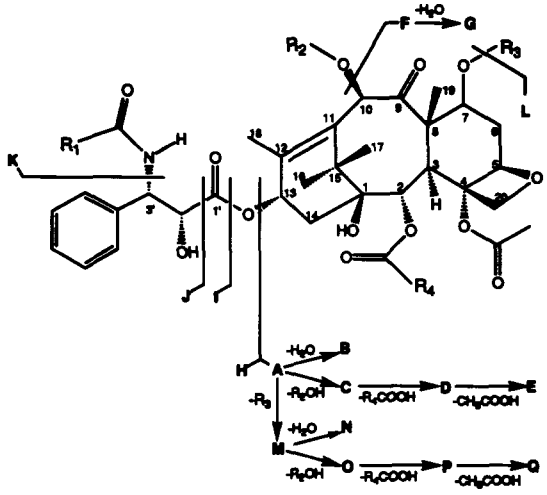

FIGURE 1. General taxane structure and scheme of ms/ms cid fragmentation. In paclitaxel, $R_1=C_6H_5$, $R_2=CH_3CO$, $R_3=H$, and $R_4=C_6H_5$.

and other taxanes have been reported (18–23). Our studies of hplc profiles of extracts and process intermediates from *Taxus brevifolia* and *T. baccata* (Taxaceae) biomass indicated many taxane compounds for which identification was desirable, and the present analytical study of these taxanes has utilized a procedure that is based on previous methods for the rapid and systematic elucidation of drug metabolites in physiological samples (24) and natural products in crude extracts (16). Analyses combined optimized hplc separation conditions on-line with an ionspray ms interface to obtain full-scan mass spectra and tandem mass spectra. In this way, structural and substructural data for trace taxane components in mixtures were obtained rapidly and systematically without prior fractionation.

Due to the high sensitivity of mass spectrometry this method was particularly advantageous for application to samples of limited quantity, a situation frequently encountered in pharmaceutical discovery and development research. In addition, lc/ms and lc/ms/ms techniques facilitated the rapid analysis of samples based on the integration of bench-scale mixture analysis methodology (scale-up, fractionation and individual spectroscopic analysis) into one on-line instrumental technique. Using this methodology, detailed structural information was typically obtained for ten taxanes in a complex mixture in less than one day.

RESULTS AND DISCUSSION

Use of an integrated analytical system incorporating hplc separation, uv spectroscopy, ms, and ms/ms rapidly provided profiles and substructure information useful for compound identification. On-line hplc chromatographic separation afforded a profile of the components in the sample and their relative concentrations. Hplc provided a reproducible relative retention (RR_t, relative to paclitaxel) time using standardized hplc conditions for the development of a taxane database and the transfer of data to collaborating laboratories. The reversed-phase hplc conditions also provided a general measure of lipophilicity of each compound, useful for interpretation of substructural differences between related taxanes. RR_t values were reproducible to approximately $\pm$ 5%. Hplc condition I provided good general separation conditions, especially for more lipophilic taxanes, and hplc condition II provided better separation of earlier eluting (less lipophilic) taxanes. Uv spectra collected on-line provided evidence for general classification and substructures of each compound.

Generally, taxanes produced uv spectra with absorption maxima at approximately 227 and 250 nm. The ionspray (nebulizer assisted electrospray) interface (25) generated reproducible, abundant adduct ions $(M+NH_4)^+$, which were the base peaks for all of the taxanes. The $(M+NH_4)^+$ ions provided reliable mol wt confirmation from full-scan mass spectrometry, as well as abundant ion current, which is favorable for trace ms/ms analysis.

Chromatographic resolution of co-eluting or unresolved components was not required to obtain product ion data for structural analysis, due to the mass-resolving capability of mass spectrometry. Product ion spectra provided evidence for the substructures of each natural product. The fragmentation patterns of paclitaxel and other well-characterized taxanes were used as substructural "templates" for interpretation of the structures of unknown taxanes by the association of specific product ions and neutral losses with specific substructures. This ms/ms comparative method is based on the premise that most of the compounds of interest contained in the extract samples would be expected to retain much of the original paclitaxel structure, such as the taxane core substructure. Therefore, paclitaxel-related compounds would be expected to undergo similar fragmentations to paclitaxel and other taxane standards. Common ms/ms

product ions and neutral losses observed in paclitaxel and in unknown taxanes were evidence for common substructures and differences were indicative of the variance in those substructures.

Structural data for 18 commonly observed taxanes from various samples are provided in Tables 1 and 2, including chromatographic behavior and mol wt, listed in Table 1, as well as prominent ions in the ms/ms product ion spectra, listed in Table 2. Due to the lack of either a basic or acidic functionality, taxanes characteristically produce abundant adduct ions $(M+NH_4)^+$ when ammonium ions are present in the mobile phase. The mass-resolving capability of mass spectrometry permits independent ms analysis of co-eluting or unresolved compounds with different mol wts, thus, producing an "lc/ms profile" of the sample, including both chromatographic behavior and mol wt dimensions. Frequently, lc/ms reveals a greater number of components (which ionize effectively under ionspray conditions) in a sample than hplc with uv detection alone, such as unresolved components and those not sensitive to uv detection. Thus, this integrated method for taxanes rapidly provides a multi-dimensional profile of the components in a complex extract, containing detailed profiles of the chromatographic behavior, uv spectrum, full-scan mass spectrum, and ms/ms product ion spectrum of individual mixture components.

A representative hplc profile chromatogram with uv detection (230 nm) of a process penultimate lot of paclitaxel from *T. brevifolia* bark is shown in Figure 2A. A large

TABLE 1. Taxanes Identified Using Lc/ms Profiling and Lc/ms/ms Substructural Analysis.

Proposed Structure	No.	RR_t[a]	Mol wt[b]	R_1[c]	R_2[c]	R_3[c]
10-Deacetylbaccatin III[d]	1	0.08 [1.00]	544	—	H	H
Baccatin III[d]	2	0.16	586	—	CH_3CO	H
7-Xylosyl-10-deacetyl-cephalomannine	3	0.22	921	C_4H_7	H	β-Xylose
7-Xylosyl-10-deacetylpaclitaxel	4	0.29	943	C_6H_5	H	β-Xylose
10-Deacetylcephalomannine	5	0.32 [1.70]	789	C_4H_7	H	H
7-Xylosyl-10-deacetyltaxol C	6	0.35	937	C_5H_{11}	H	β-Xylose
7-Xylosyl-cephalomannine	7	0.35	963	C_4H_7	CH_3CO	β-Xylose
10-Deacetylpaclitaxel[d]	8	0.44 [1.78]	811	C_6H_5	H	H
Paclitaxel isomer with C-3–C-11 bridge[e]	9	0.48	853	C_6H_5	CH_3CO	H
7-Xylosyl-paclitaxel[d]	10	0.49	985	C_6H_5	CH_3CO	β-Xylose
10-Deacetyltaxol C	11	0.56 [1.84]	805	C_5H_{11}	H	H
10-Deacetyl-7-*epi*-cephalomannine	12	0.60	789	C_4H_7	H	H
7-Xylosyl-taxol C	13	0.62	979	C_5H_{11}	CH_3CO	β-Xylose
Cephalomannine[d]	14	0.72	831	C_4H_7	CH_3CO	H
10-Deacetyl-7-*epi*-paclitaxel[d]	15	0.82	811	C_6H_5	H	H
Paclitaxel[d]	16	1.00	853	C_6H_5	CH_3CO	H
Taxol C	17	1.05	847	C_5H_{11}	CH_3CO	H
7-*epi*-Paclitaxel[d]	18	1.17	853	C_6H_5	CH_3CO	H
7-Triethylsilyl-paclitaxel[f]	19	1.38	967	C_6H_5	CH_3CO	$Si(C_2H_5)_3$

[a]Hplc relative retention time compared to paclitaxel (RR_t = 1.00, retention time = 30.0 min) using hplc condition I or [condition II].

[b]Molecular weight.

[c]Refer to Figure 1 for the general taxane structure including R groups. R_4 is C_6H_5 for all of these compounds. Position of attachment to the core is proposed based on comparison or confirmation with authentic taxane templates.

[d]Confirmed using authentic standards by RR_t, mol wt, and ms/ms product ion spectra.

[e]Confirmed by collecting fractions at this retention time and analyzing by ^1H nmr (9).

[f]Observed in semi-synthetic paclitaxel intermediates synthesized from 10-deacetylbaccatin III.

TABLE 2. Ms/ms Product Ion Spectra of $(M+NH_4)^+$ Ions of Taxanes and Interpretations.[a]

Proposed Structure	Fragment Peak (*m/z*)														
	$(M+NH_4)^+$	$(M+H)^+$	A (M)	B (N)	C (O)	D (P)	E (Q)	F	G	H	I	J	K	L	Others
10-Deacetylbaccatin III	562	545	527	509	467	387	327	527	509						177, 299, 345, 449, 467
Baccatin III	604	587			509		327	527	509						299, 345, 405, 449
7-Xylosyl-10-deacetylcephalomannine	939	922	659 (527)	641 (509)		(327)				264		218		$-H_2O$ 772	
7-Xylosyl-10-deacetylpaclitaxel	961	944	659 (527)	641 (509)		(387)	(327)	926		286				$-H_2O$ 794	
10-Deacetylcephalomannine	807	790	527	509			327			264	246				309, 369
7-Xylosyl-10-deacetyltaxol C	955	938	659 (527)	641 (509)				920		280				$-H_2O$ 788	
7-Xylosylcephalomannine	981	964	701 (569)		641 (509)					264	246			832	
10-Deacetylpaclitaxel	829	812	527	509			327	794		286	268	240	122		309, 655
Paclitaxel isomer with C-3–C-11 bridge	871	854			509	387	327	794	776	286	268	240	122		345, 390, 449, 467, 672
7-Xylosyl-paclitaxel	1003	986	701 (569)	(551)	641 (509)	(387)	(327)	926	906	286	268	240		854	
10-Deacetyl-7-*epi*-cephalomannine	807	790	527	509						264			100		83
7-Xylosyl-taxol C	997	980	701 (569)	(551)	641 (509)					280	262	234		848	788
Cephalomannine	849	832	569	551	509	387	327	772	754	264	246				309
10-Deacetyl-7-*epi*-paclitaxel	829	812	527	509			327	794	776	286	268	240	122		
Paclitaxel	871	854	569	551	509	387	327	794	776	286	268	240	122	836	309, 491, 655
Taxol C	865	848	569	551	509		327		770	280	262				
7-*epi*-Paclitaxel	871	854	569	551	509	387	327	794	776	286	268	240	122		309
7-Triethylsilyl-paclitaxel	985	968	683	665	623	501	441	908	890	286	268				309, 327

[a]See Figure 1 for generalized ms/ms fragmentations A–L.

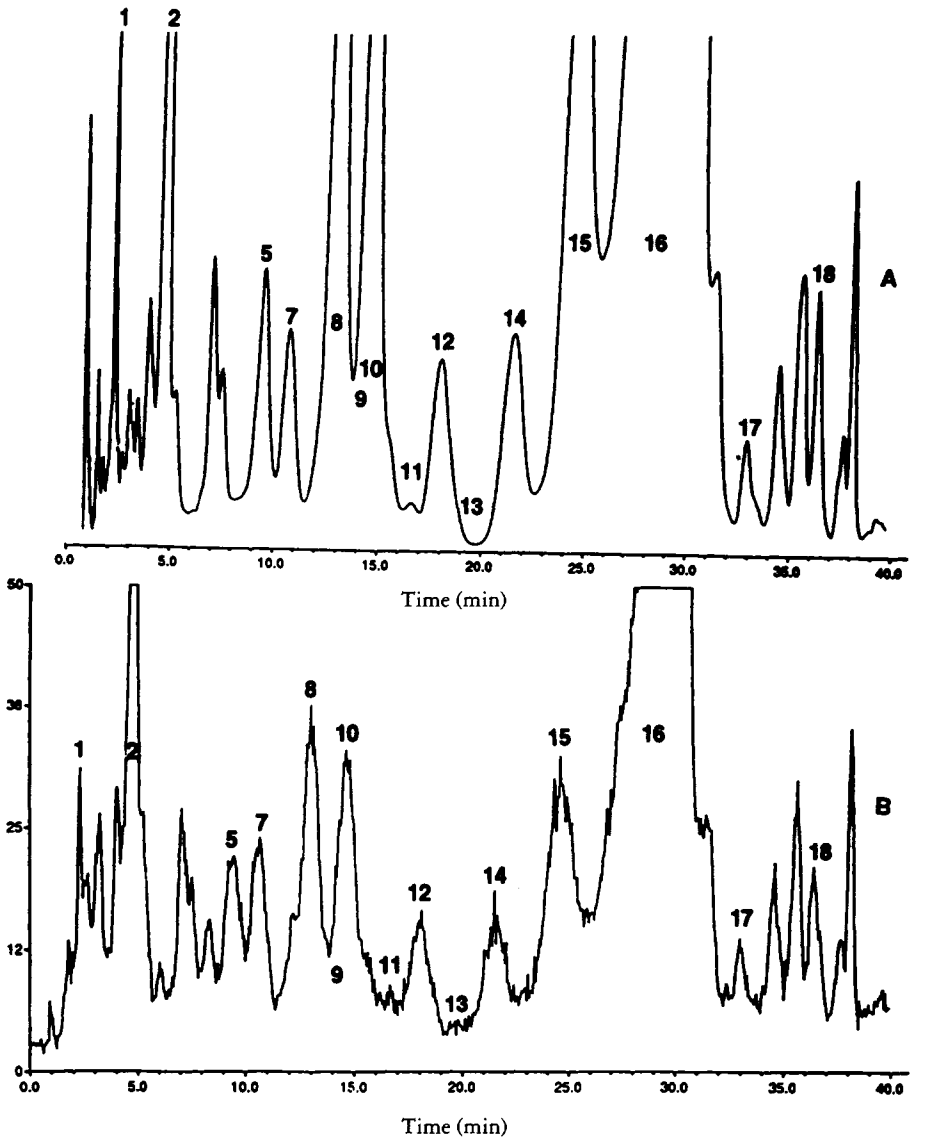

FIGURE 2. Hplc chromatograms using condition I, of a process intermediate sample from the isolation of paclitaxel bulk drug. (A) Lc/uv profile chromatogram (230 nm). (B) Lc/ms (ionspray) profile total ion current chromatogram.

number of natural products from *T. brevifolia* are chromatographically separated in this sample, which is less complicated than typical crude biomass extracts. A comparable lc/ms profile chromatogram (total ion current) of the sample, obtained simultaneously with the lc/uv profile, is shown in Figure 2B, indicating the comparability of the ionspray mass spectrometry response with uv detection. The mol wt of each component was obtained on-line from the full-scan ionspray mass spectrum at its retention time as illustrated for 7-xylosyl-paclitaxel (*RR*, 0.49) in Figure 3. This information was obtained from the low ionization energy ionspray interface despite other unresolved sample components which produced other ions (*m/z* 502–690) seen in Figure 3.

Mass chromatograms (extracted ion current profiles) corresponding to the $(M+NH_4)^+$ ions of selected components (Figure 4), indicate the specificity of lc/ms for mol wt

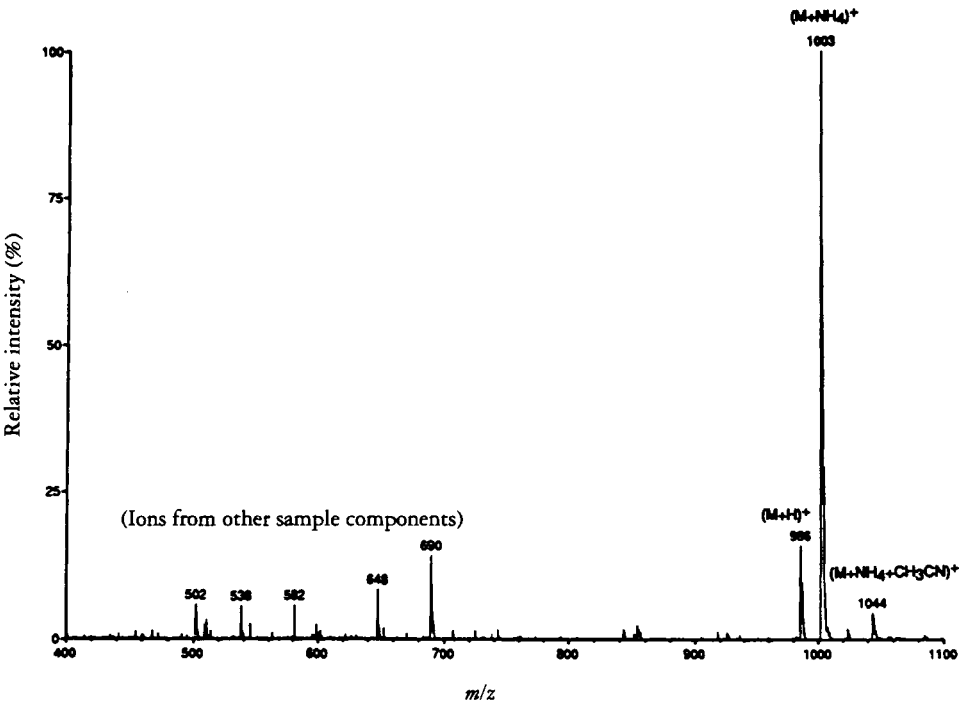

FIGURE 3. Full-scan mass spectrum of 7-xylosyl-paclitaxel (mol wt 985) at *RR*, 0.49 in the process intermediate sample, indicating the correspondence of ions observed to molecular weight.

differentiation and determination of taxane components in the complex *Taxus*-derived mixture. The difference between the mol wt of a taxane and paclitaxel is indicative of the substructural differences between the taxane and paclitaxel. For example, comparison of the full-scan ionspray mass spectrum of 10-deacetylpaclitaxel (Figure 5A) and paclitaxel (Figure 5B) demonstrates a mol wt difference of 42 daltons, which is commonly indicative in taxanes of an acetyl substructural difference, or may be due to another substructural difference, such as C_3H_7.

Following the determination of the mol wt of each component in the initial hplc separation, the sample was again subjected to hplc separation, during which the product ion spectrum of the $(M+NH_4)^+$ ion of each compound was obtained at its retention time, providing specific fragmentation data for each component. The fragmentation of paclitaxel has been previously described (19) and is shown in Figure 1. The structure of each taxane was proposed based on comparison of product ions and neutral losses observed in the product ion spectrum with the product ions and neutral losses associated with specific substructures of paclitaxel (Figure 6). Furthermore, according to the nitrogen rule, the even *m/z* ratio of the product ions associated with the side-chain is generally indicative of an odd number (1) of nitrogen atoms in the side-chain fragment ion (when fragmentation does not result from free radical or rearrangement mechanisms), indicating the presence or absence of the side-chain amide. (Low energy cid of even-electron species generally results in loss of small neutral molecules). The positions of attachment of these substructures to the paclitaxel core were not definitively determined using ms/ms, but were proposed in Table 1 on the basis of spectral comparison to authentic taxane standards.

An example of taxane substructural analysis using lc/ms/ms is illustrated for 7-xylosyl-10-deacetylpaclitaxel, shown in Figure 7. Comparison to the paclitaxel ms/ms

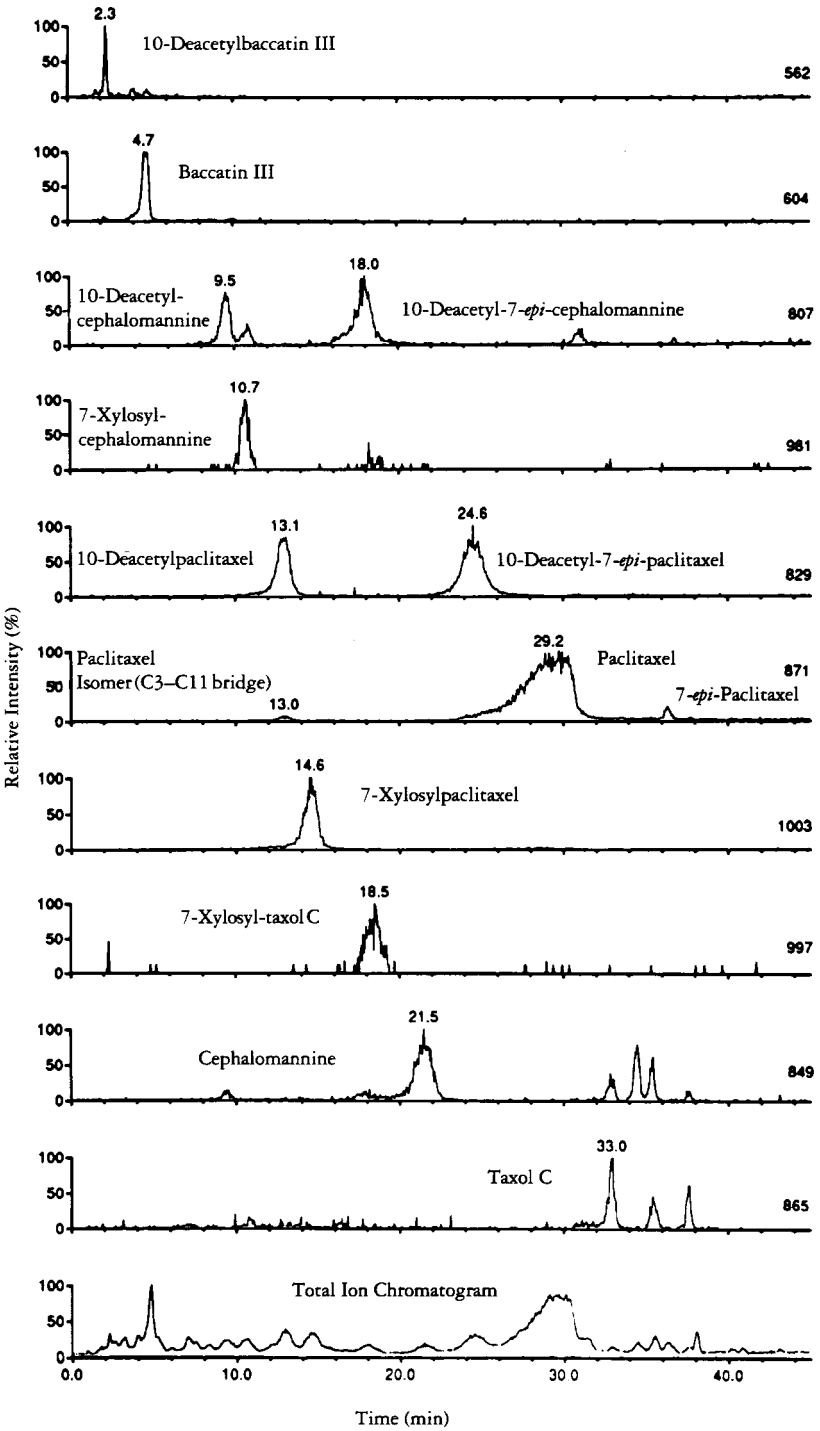

FIGURE 4. Lc/ms mass chromatograms (extracted ion current profiles) of selected taxanes, at the *m/z* ratio of their $(M+NH_4)^+$ ions, in the process intermediate sample shown in Figure 2. The *m/z* ratio of the mass chromatograms plotted is shown at the right of each trace. Note the "double peaks" associated with epimers of 10-deacetylcephalomannine, 10-deacetylpaclitaxel, and paclitaxel. Unlabeled peaks in some mass chromatograms correspond to isobaric compounds or compounds producing adduct ions of the same *m/z* value, which are distinguished by their ms/ms product ion spectra from the labeled taxanes.

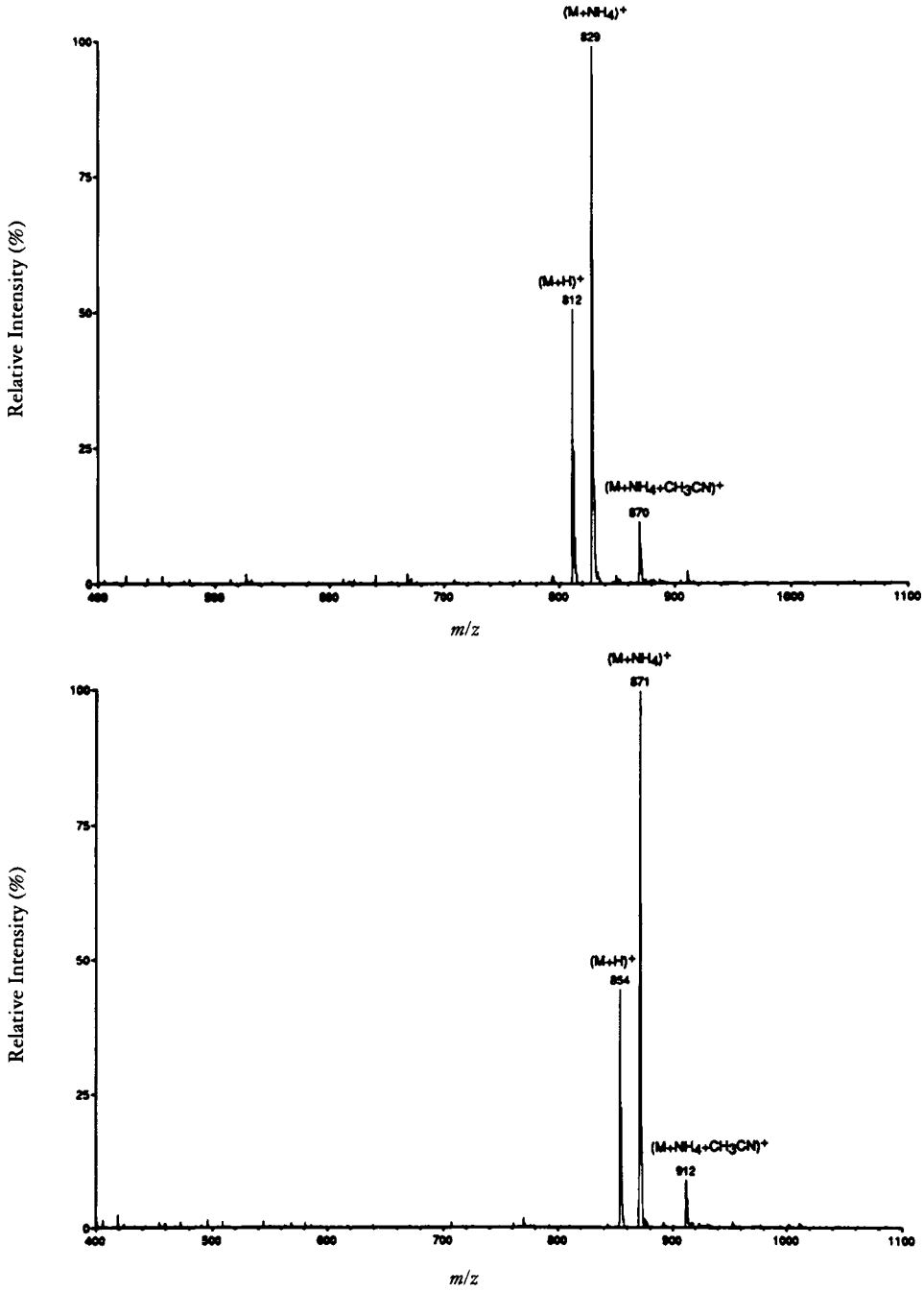

FIGURE 5. Full-scan ionspray mass spectra of: (A) 10-deacetylpaclitaxel (mol wt 811) and (B) paclitaxel (mol wt 853), in the process intermediate sample, indicating a mol wt difference of 42 daltons, which is commonly indicative of an acetyl substructure in taxanes.

substructural template (Figure 6) indicated the structure shown in Figure 7. The product ion at *m/z* 527 differs from the paclitaxel core substructure product ion at *m/z* 569 by 42 daltons, indicating one fewer acetyl group on the core substructure than for paclitaxel. The presence of the product ion at *m/z* 509 is diagnostic of the deacetylated paclitaxel

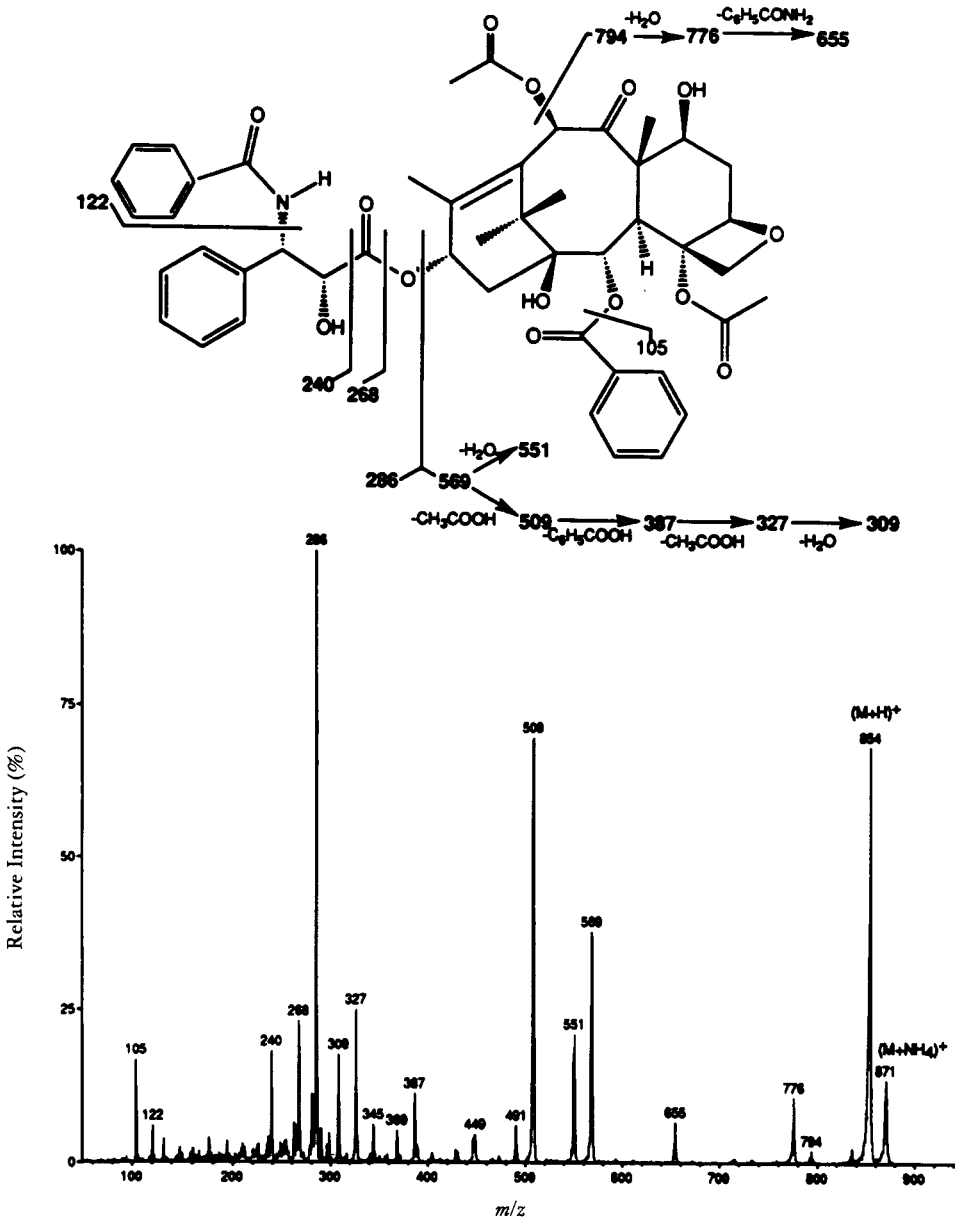

FIGURE 6. Product ion spectrum of the *m/z* 871 $(M+NH_4)^+$ ion of paclitaxel used as a template for structural analysis of related taxanes. Product ions, neutral losses and their correspondence to specific substructures are indicated.

core substructure. The observed mol wt of this taxane (943 daltons) differs from paclitaxel by 90 daltons. Combining this 90-dalton difference with the 42-dalton difference, indicated above as due to deacetylation of the paclitaxel core substructure, yields a total difference of 132 daltons, characteristic of a sugar substructure, such as xylose, attached to a core hydroxyl. The neutral loss of 150 daltons from the $(M+H)^+$ ion to the ion at *m/z* 794 indicates the loss of the neutral sugar molecule. The attachment of this sugar substructure to the core substructure was indicated by the neutral loss of 150 daltons from the ion at *m/z* 659 (the deacetylated core substructure with the sugar

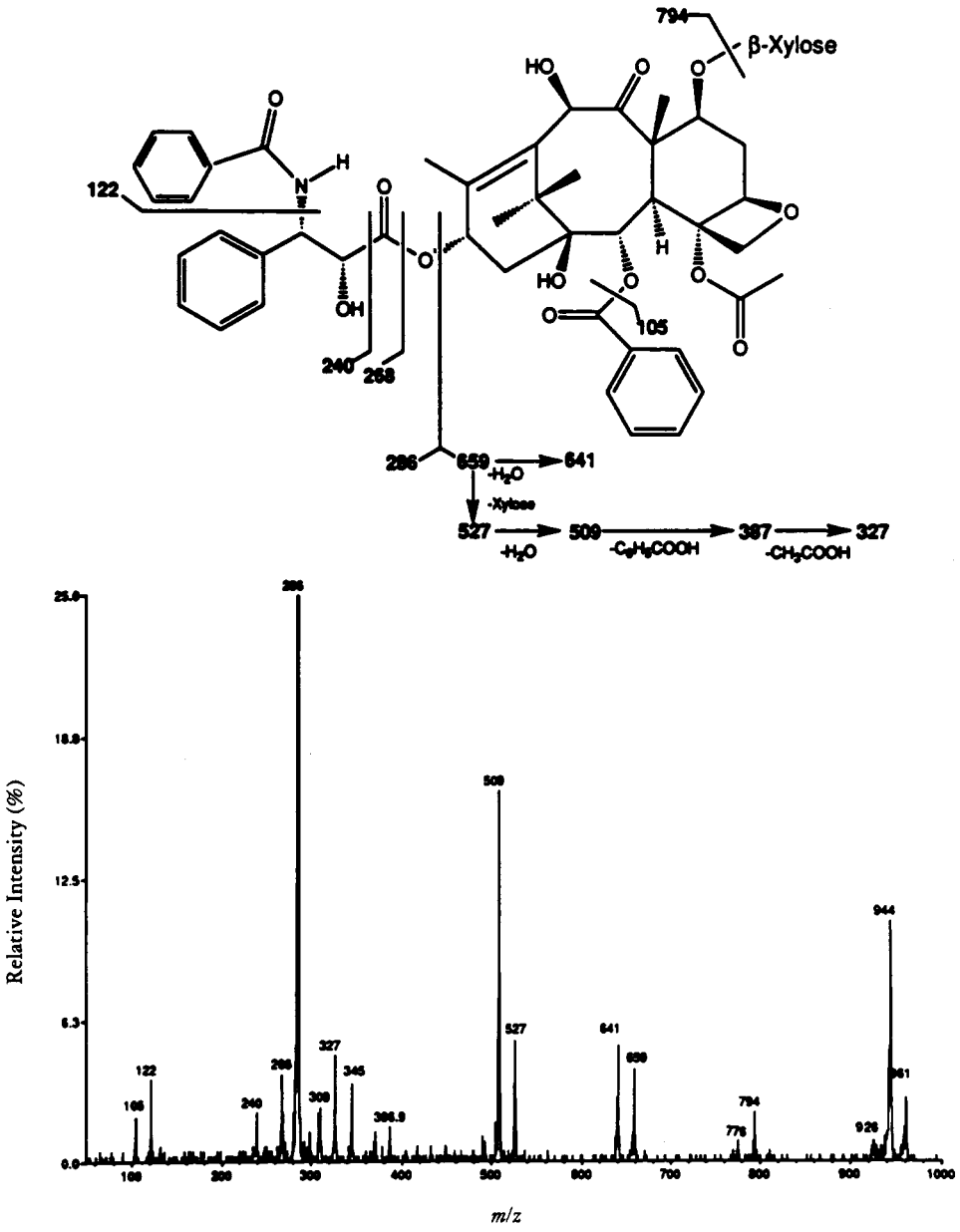

FIGURE 7. Product ion spectrum of the ion at *m/z* 961 $(M+NH_4)^+$ of 7-xylosyl-10-deacetylpaclitaxel at RR_t 0.29 in a *T. brevifolia* extract.

attached) to the ion at *m/z* 509 (the deacetylated core substructure of paclitaxel). The ions at *m/z* 286, 240, and 122 are diagnostic of the paclitaxel side-chain. The neutral loss of 18 daltons from the ion at *m/z* 944 to the ion at *m/z* 926 is indicative of a hydroxyl group. The neutral loss of 60 daltons from the ion at *m/z* 387 to the ion at *m/z* 327 is indicative of an acetyl group. Thus, the structure of the taxane at RR_t 0.29 is proposed to be xylosyl-deacetylpaclitaxel. Although these results can not confirm that the sugar is xylose or the position of its attachment, they are consistent with the structure previously reported as 7-xylosyl-10-deacetylpaclitaxel (7), as well as common sites of xylose attachment and deacetylation in previously reported taxanes (1, 3, 5–8, 10–13).

The observed taxanes incorporate several consistent structural variations from paclitaxel. Nearly all of the compounds contain the characteristic paclitaxel core substructure as indicated by the product ion at *m/z* 509 with variations due to substructures attached to this characteristic substructure. Many of these taxanes have a side-chain similar to paclitaxel, with variation occurring in the amide terminating the side-chain. These terminal amide variations are readily indicated by product ions differing from the characteristic side-chain ions of paclitaxel (e.g., *m/z* 286) by values diagnostic of specific substructures. For example, the side-chain ion of taxol C was observed at *m/z* 280, indicative of the 6-dalton difference between the phenyl substructure (77 daltons) and the pentyl substructure (71 daltons). The even *m/z* ratio indicated that an amide was present in the unknown, deduced via the nitrogen rule. Thus, comparison with the paclitaxel "template" indicated structural differences beyond the position of the amide group in the side-chain substructure. Modifications beyond the amide group are also consistent with previous literature reports of taxanes (1–8, 10–14). The product ion spectra of taxanes altered in the terminal amide also indicated characteristic fragmentations in the side-chain (J and K in Table 2) further defining the position of the variation compared to paclitaxel. The increasing reversed-phase hplc retention with increasing mol wt of compound in this series is consistent with the increasing lipophilicity of the side-chain as the chain-length increases.

A consistent pattern of two isobaric components producing two chromatographic peaks was commonly observed in the mass chromatograms of *Taxus* extracts. The identities of these peaks were confirmed using authentic standards (e.g., paclitaxel and 7-*epi*-paclitaxel, 10-deacetylpaclitaxel and 10-deacetyl-7-*epi*-paclitaxel) as epimers at C-7. The later eluting isomer had the 7β- configuration in these standards. Figure 4 clearly illustrates the "double peaks" associated with epimers of 10-deacetylcephalomannine, 10-deacetylpaclitaxel, and paclitaxel. (Unlabeled peaks in some of the mass chromatograms correspond to non-isomeric compounds producing adduct ions [e.g., $(M+Na)^+$, $(M+H)^+$, $(M+NH_4+CH_3CN)^+$] having the same *m/z* ratio as the taxanes of interest and are distinguishable by mol wt or product ions.) This pattern of C-7 epimer elution order was logically extended to the proposal of structures for other isobaric pairs. C-7 epimers produced product ion spectra containing the same fragment ions, but often differing in the relative abundance of some product ions.

Many taxanes incorporate a sugar substructure attached to the taxane core substructure, which have been proposed as 7β-xylosyl taxanes. This is based on previously reported taxanes incorporating β-xylose at C-7 (7). In addition, an authentic standard of 7β-xylosyl-paclitaxel was used to confirm the structural assignment of the taxane observed at RR_t 0.49 (based on RR_s, mol wt and ms/ms product ion spectrum). If larger quantities of purified taxanes are prepared, the position of attachment and conformation of the sugar could be further studied using nmr or single-crystal X-ray analysis. The xylosylated analogues are more polar than the corresponding taxane aglycone and, thus, elute at an earlier retention time than the aglycone using the standardized reversed-phase hplc conditions described earlier.

Another common structural variation among taxanes observed in this study is the degree of acetylation of the core substructure. Standards of 10-deacetylpaclitaxel, 10-deacetyl-7-*epi*-paclitaxel and 10-deacetylbaccatin III confirmed a consistent pattern of absence of acetylation at C-10. This trend was logically extended to propose the structures of other deacetylated taxanes. The increased polarity of the deacetylated analogues resulted in earlier elution times using reversed-phase chromatography compared to their respective acetylated analogues (e.g., 10-deacetylpaclitaxel vs. paclitaxel).

Structural data for 18 taxanes are compiled in Tables 1 and 2. In addition to the data presented here, the structural assignments are all supported by comparable structures in

the literature. Many of the compounds in the Tables were also confirmed by comparing the uv spectra, chromatographic RR_t, mol wt, and ms/ms product ion spectra of the taxane and a corresponding authentic taxane standard analyzed under the same conditions. Compounds confirmed using this systematic approach are noted in Tables 1 and 2.

The application of rapid and systematic lc/ms profiling and lc/ms/ms substructural analysis protocols to the assignment of structure of taxanes in *T. brevifolia* and *T. baccata* biomass extracts, process intermediates, and bulk paclitaxel drug has therefore resulted in a detailed taxane structural database, containing characteristic chromatographic (RR_t) and mass spectrometric (mol wt, product ions) data indicative of structure. This database has been utilized for the rapid identification of known taxanes and proposal of structures of novel taxanes found in trace quantities (E.H. Kerns, K.J. Volk, S.E. Hill, and M.S. Lee, unpublished data).

The use of standardized hplc conditions provided reproducible chromatographic data for the rapid identification of previously known compounds. The lc/ms profile provided mol wt data complementary to the lc/uv profile, thus extending the detail of data obtained in mixture profiling to mol wt, a powerful, specific element in taxane analysis. In addition, incorporation of lc/ms/ms methodology added substructural data for trace (nanogram to microgram quantity) components without prior fractionation.

The lc/ms profiling and lc/ms/ms substructural analysis methodologies utilized herein for taxanes are very useful in natural products research and development, especially for unfractionated samples containing trace components. In many pharmaceutical research studies, the amount of sample is limited or the process of scale-up and purification is expensive and time-consuming, and associated processes may result in compound degradation. Thus, methods incorporating lc/ms and lc/ms/ms techniques are powerful strategies for the rapid identification and confirmation of known natural products, exploration for potentially novel compounds, and as a primary front-line technique in a multidisciplinary structure elucidation strategy utilizing analytical techniques according to the available quantity and purity of a sample.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Samples from evaporated biomass MeOH extractions, process intermediate fractions and paclitaxel bulk drug from *T. brevifolia* or *T. baccata* (Hauser Chemical Research) were dissolved at a concentration of 10 mg/ml in solvent consisting of hplc grade MeOH (Fisher) modified with 0.05% (v/v) glacial HOAc (Fisher). A 20-µl aliquot of this solution was injected onto the hplc column. Authentic standards of 10-deacetylbaccatin III, baccatin III, 10-deacetylpaclitaxel, 7-xylosyl-paclitaxel, cephalomannine, 10-deacetyl-7-*epi*-paclitaxel, paclitaxel, and 7-*epi*-paclitaxel were obtained from Bristol-Myers Squibb Co.

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY.—Two hplc chromatographic conditions were used for hplc profiling of samples. Condition I[1] utilized a Chromegabond pentafluorophenyl reversed-phase column (4.0 mm×250 mm×5 µm, 60 Å, ES Industries) with a mobile phase of 65/35 buffer (2 mM ammonium acetate, pH 6.9)/CH_3CN held isocratic for 26 min, followed by a linear gradient to 20/80 buffer/CH_3CN over 25 min at a flow rate of 2.0 ml/min (26). Condition II[2] utilized a Chromegabond phenyl reversed-phase column (4.6 mm×150 mm×3 µm, 60 Å, ES Industries) with a mobile phase of 90/10 buffer (2 mM ammonium acetate, pH 6.9)/CH_3CN held isocratic for 2.0 min, followed by a linear gradient to 39/61 buffer/CH_3CN over 30 min at a flow rate of 2.0 ml/min. Mobile phase was delivered by a Perkin-Elmer Series 410 pump. Uv spectra and chromatograms were obtained using a Perkin-Elmer LC-235 photodiode array detector on-line between the hplc column and the mass spectrometer. Chromatograms were obtained at 230 nm.

Mass spectrometry analysis was performed using a Sciex API III tandem quadrupole mass spectrometer equipped with an ionspray lc/ms interface. Hplc column eluents were directed into the uv detector, followed

[1]B. Floor, personal communication of hplc condition I.

[2]V. Warren, personal communication of hplc condition II.

by introduction into the mass spectrometer using a 20:1 split. The ionspray tip was operated at +5200 V. Full-scan mass spectra were obtained while continuously scanning from *m/z* 300 to *m/z* 1200 with a step size of 0.4 mass units and a dwell time of 1.5 msec. Ms/ms product ion spectra were obtained by selection of the adduct ions $(M+NH_4)^+$ of each analyte in the first mass separation stage of the ms/ms system at their hplc retention time, followed by collision-induced dissociation (cid) with argon collision gas at 450×10^{12} atoms/cm^2 and a collision energy of 70 eV, while scanning the final mass separation stage from *m/z* 50 to approximately *m/z* 20 greater than the parent $(M+NH_4)^+$ ions to record the product ions. Mass spectral information was readily obtained for 100-200 ng of each component in the mixture.

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LITERATURE CITED

1. M. Suffness and G.A. Cordell, in: "The Alkaloids. Chemistry and Pharmacology." Ed. by A. Brossi, Academic Press, New York, 1985, Vol. 35, p. 3.
2. P.B. Schiff, J. Fant, and S.B. Horwitz, *Nature*, **277**, 665 (1979).
3. J. Parness, D.G.I. Kingston, R.G. Powell, C. Harracksingh, and S.B. Horwitz, *Biochem. Biophys. Res. Commun.*, **105**, 1082 (1982).
4. M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, and A.T. McPhail, *J. Am. Chem. Soc.*, **93**, 2325 (1971).
5. R.W. Miller, *J. Nat. Prod.*, **43**, 425 (1980).
6. J.L. McLaughlin, R.W. Miller, R.G. Powell, and C.R. Smith, Jr., *J. Nat. Prod.*, **44**, 312 (1981).
7. V. Senilh, S. Blechert, M. Colin, D. Guénard, F. Picot, P. Potier, and P. Varenne, *J. Nat. Prod.*, **47**, 131 (1984).
8. S. Blechert, in: "The Alkaloids. Chemistry and Pharmacology." Ed. by A. Brossi, Academic Press, New York, 1990, Vol. 39, p. 195.
9. S.H. Chen, C.M. Combs, S.E. Hill, V. Farina, and T.W. Doyle, *Tetrahedron Lett.*, **33**, 7679 (1992).
10. D.G.I. Kingston, *Pharmac. Ther.*, **52**, 1 (1991).
11. D.P. Della Casa de Marcano and T.G. Halsall, *J. Chem. Soc., Chem. Commun.*, **9**, 365 (1975).
12. R.W. Miller, R.G. Powell, and C.R. Smith, Jr., *J. Org. Chem.*, **46**, 1469 (1981).
13. L. Barboni, P. Gariboldi, E. Torrengiani, G. Appendino, B. Gabetta, G. Zini, and E. Bombardelli, *Phytochemistry*, **33**, 145 (1993).
14. J.V. Johnson and R.A. Yost, *Anal. Chem.*, **57**, 758A (1985).
15. 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.
16. M.S. Lee, D.J. Hook, E.H. Kerns, K.J. Volk, and I.E. Rosenberg, *Biol. Mass Spectrom.*, **22**, 84 (1993).
17. A. Ranasinghe, J.D. Sweatlock, and R.G. Cooks, *J. Nat. Prod.*, **56**, 552 (1993).
18. S.H. Hoke II, J.M. Wood, R.G. Cooks, X.H. Li, and C.-J. Chang, *Anal. Chem.*, **64**, 2113 (1992).
19. T.D. McClure, K. Schram, and M.L. Reimer, *J. Am. Soc. Spectrom.*, **3**, 672 (1992).
20. P.K.S. Blay, P. Thibault, N. Thiberge, B. Kiecken, A. Leburn, and C. Mercure, *J. Rapid Commun. Mass Spectrom.*, **7**, 626 (1993).
21. S.O.K. Auriola, A. Lepisto, T. Naaranlahti, and S.P. Lapinjoki, *J. Chromatogr.*, **594**, 153 (1992).
22. F. Bitsch, W. Ma, F. Macdonald, M. Neider, and C.H. Shackleton, *J. Chromatogr.*, **615**, 273 (1993).
23. F. Bitsch, C.H.L. Shackelton, W. Ma, G. Park, and M. Nieder, *J. Rapid Commun. Mass Spectrom.*, **7**, 891 (1993).
24. M.S. Lee and R.A. Yost, *Biomed. Env. Mass Spectrom.*, **15**, 193 (1988).
25. A. Bruins, T.R. Covey, and J.D. Henion, *Anal. Chem.*, **59**, 2642 (1987).
26. S.L. Richheimer, D.M. Tinnermeier, and D.W. Timmons, *Anal. Chem.*, **64**, 2323 (1992).

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