NEW TERTIARY ALKALOIDS OF *STRYCHNOS DECUSSATA*

WENCHE N. A. ROLFSEN* and AJIBOLA A. OLANIYI[1]

Department of Pharmacognosy, Faculty of Pharmacy, Box 579, Biomedical Centre, University of Uppsala, S-751 23 Uppsala, Sweden

and

PETER J. HYLANDS

Department of Pharmacy, Chelsea College, Manresa Road, University of London, London, S.W.3, 6LX, England

ABSTRACT.—From the stem bark of *Strychnos decussata* (Pappe) Gilg (Loganiaceae), five tertiary indole alkaloids have been isolated and identified.

Previously known alkaloids, but new to this plant, were: akagerine (**1**) and 17-*O*-methylakagerine (**2**), while alkaloids newly found in nature were 10-hydroxy-21-*O*-methylkribine (**3a**), 10-hydroxyepi-21-*O*-methylkribine (**3b**) and 10-hydroxy-17-*O*-methylakagerine (**4**). Structures have been ascribed to the new compounds on the basis of their spectral, particularly nmr, data. The pharmacological activity of the alkaloids is also discussed.

Strychnos decussata is a small tree or a shrub distributed mainly in East and South Africa and Madagascar. During screening of some East African *Strychnos* species for muscle-relaxant and convulsive effects (1), it was found that the alkaloid fraction of the stem and root barks of *S. decussata* had a strong muscle-relaxant effect, which was most pronounced in the chloroform fraction. It was, therefore, considered of interest to locate the compounds responsible for the pharmacological activity of this fraction. In the course of our search for these compounds, five of the indolic tertiary bases, three of which are new, were isolated from the stem bark and this report describes their structural elucidation. In a recent publication, Petitjean *et al.* (2) described the isolation of a gluco-alkaloid from the leaves of this plant; however, this alkaloid has not been found in the stem bark in the present work.

RESULTS AND DISCUSSION

CHEMICAL.—The plant material was extracted and chromatographed as described in the Experimental section. The fractions collected from column chromatography were purified by repeated preparative tlc on silica gel to afford the pure alkaloids. By this procedure, five of the major alkaloids were isolated in sufficient quantities to allow complete identification and preliminary testing for the biological activities. Work is in progress on the isolation and structural elucidation of the remaining alkaloids. The structures of the five alkaloids are given in fig. 1.

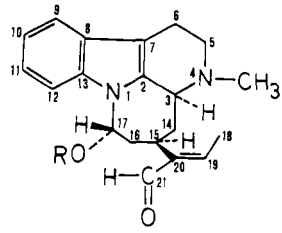
One of the alkaloids was shown to be identical with akagerine (**1**) (3–5) from spectral studies (uv, ir, nmr and ms) and by comparison with an authentic sample (co-tlc).

The second alkaloid was identified as 17-*O*-methylakagerine (**2**) (5) from spectral data (uv, ir, nmr and ms) and by comparison with an authentic sample (mmp, co-tlc).

*To whom correspondence should be addressed.

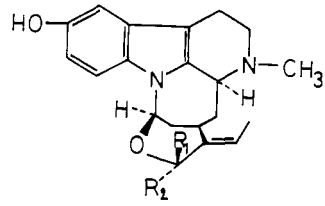
[1]Present address: Dept. of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ife, Ile-Ife, Nigeria.

The third (**3a**) and fourth (**3b**) alkaloids both have the molecular ion at m/z[2] 354, analyzing for $C_{21}H_{26}N_2O_3$. The two alkaloids showed very similar spectral data, much like 21-*O*-methylkribine (**5a**) and epi-21-*O*-methylkribine (**5b**), recently isolated from *S. dale* and *S. elaeocarpa* (5). However, the presence of absorption peaks at 3360 and 3400 cm^{-1}, respectively, in the ir spectra of (**3a**) and (**3b**) (these peaks were not present in the corresponding spectra of (**5a**) and (**5b**)) and the alkali induced bathochromic shifts in the uv spectra of (**3a**) and (**3b**), indicated the presence of a phenolic group in each of the two compounds. Comparison of the ms of these two substances with those of (**5a**) and (**5b**), revealed further similarities with a constant difference of 16 mass units for the fragments containing the aromatic moiety. Further evidence for the presence of a phenolic group was



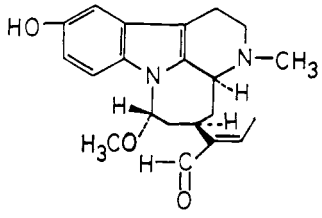
1; R=H

2; R=CH_3

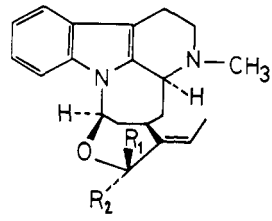


3a; R_1=OCH_3,R_2=H

3b; R_1=H,R_2=OCH_3



4



5a; R_1=OCH_3,R_2=H

5b; R_1=H,R_2=OCH_3

FIG. 1. Structures of alkaloids isolated from *Strychnos decussata* and other alkaloids cited in the text.

1 = akagerine
2 = 17-*O*-methylakagerine
3a = 10-hydroxy-21-*O*-methylkribine
3b = 10-hydroxy-epi-21-*O*-methylkribine
4 = 10-hydroxy-17-*O*-methylakagerine
5a = 21-*O*-methylkribine
5b = epi-21-*O*-methylkribine

provided by the broad signals observed in the ^{1}H-nmr spectra at δ5.40 in (**3a**) and δ5.13 in (**3b**) which disappeared upon the addition of D_2O. Furthermore, the signals due to the aromatic protons are shifted from δ7.60–7.04 (4H) in (**5a**) to δ7.32–6.66 (3H) in (**3a**) and from δ7.55–7.04 (4H) in (**5b**) to δ7.26–6.68 (3H) in (**3b**), indicating substitution in the aromatic nucleus. The characteristic splitting

[2]The symbol "m/z" for mass to charge ratio is preferred to "m/e", according to IUPAC recommendations on the nomenclature for mass spectrometry (15).

pattern of the aromatic signals in the ^{1}H-nmr spectra of (**3a**) and (**3b**) suggested that these compounds have the phenolic group in the 10-position. This is in accord with known hydroxylated and methylated indole derivatives (6–11).

Further confirmation of C–10 as the most likely position of the hydroxyl group was obtained from a study of the ^{13}C-nmr spectrum of (**3b**), which was compared with that of epi-21-*O*-methylkribine, (**5b**). The following substituent parameters (12) for the introduction of an hydroxyl group (α,+26.9;*O*,−12.7;*m*+1.4 and *p*,−7.3 p.p.m.) and the data from the ^{13}C-nmr spectrum of (**5b**) (5) resulted in the predicted values for the resonance position of the aromatic hydrogens in a 10-hydroxy compound are C–9 105.7, C–10 148.8, C-11 107.9 and C–12 110.5 ppm. The observed values are 103.6, 151.7, 109.9 and 112.1 ppm respectively, in close agreement with the calculated shifts. Only a 10-hydroxy compound gives figures with such good correspondence. Hence, on the basis of this and the other spectral evidence above, structures (**3a**) (10-hydroxy-21-*O*-methylkribine) and (**3b**) (10-hydroxy-epi-21-*O*-methylkribine) are established for the two new alkaloids.

Alkaloid (**4**) was characterized as 10-hydroxy-17-*O*-methylakagerine. It showed similar spectral data as 17-*O*-methylakagerine (2). However, the alkali-induced bathochromic shift in the uv spectrum and the presence of an absorption peak at 3280 cm^{-1} in the ir spectrum of (**4**), which was not present in that of (**2**), suggested the presence of a phenolic group in (**4**). The ms of (**4**) showed a molecular ion at *m/z* 354 instead of *m/z* 338 in (**2**), indicating an additional oxygen atom. The ^{1}H-nmr showed both a broad signal at δ5.35, which disappeared upon addition of D_2O, and a characteristic splitting in the signals for three (rather than four) aromatic hydrogens, in addition to a shift of the aromatic protons' resonance position from δ7.60–7.00 in (**2**) to δ7.25–6.68 in (**4**).

Thus, by reasoning analogous to the above for alkaloids (**3a**) and (**3b**), the phenolic group in (**4**) must be at C–10 (6–11); so that the alkaloid is thus 10-hydroxy-17-*O*-methylakagerine.

PHARMACOLOGICAL ACTIVITY.—In a preliminary pharmacological study in mice (5), 17-*O*-methylakagerine (**2**) was found to give clonic and tonic convulsions with a CD_{50} value at 45.3 mg/kg. In another study, akagerine (**1**) was found to have a CD_{50}-value at 50 mg/kg (4).

TABLE 1. The CD_{50} and LD_{50} values of the 10-hydroxylated alkaloids from *Strychnos decussata*.

Alkaloid tested	CD_{50}	LD_{50}	No. of animals used
10-hydroxy-21-*O*-methylkribine, **3a**....	80	90	7
10-hydroxy-epi-21-*O*-methylkribine, **3b**.	84	84	4
10-hydroxy-17-*O*-methylakagerine, **4**...	75	75	4

In our investigation for biological activity, the new hydroxy compounds **3a**, **3b** and **4** showed typical strychnine-like effects, having both clonic and tonic convulsions (the results are shown in table 1). In view of the small amount of materials available for pharmacological testing, it was not possible to draw any conclusions as to the influence of the hydroxyl substitution in the aromatic ring on the convulsive activity.

EXPERIMENTAL[3]

SOURCE AND IDENTIFICATION OF THE PLANT MATERIAL.—The stem bark of *Strychnos decussata* (Pappe) Gilg, collection number Lg10797, was collected in Korogwe, Kenya, in November 1975. The identification was confirmed by Dr. A. J. M. Leeuwenberg of the Herbarium at Wageningen, The Netherlands, where voucher specimens are kept as references.

EXTRACTION OF THE ALKALOIDS.—The ground stem bark (5 kg) was extracted twice with 1% acetic acid (10 liters) by stirring over night at room temperature. The combined filtrates were acidified to pH 2 with 5% HCl, and Mayers' reagent was added until no more precipitate was formed. The precipitate was filtered, dissolved in a mixture of acetone-methanol-water (6:2:1), and filtered again. The solution was passed through an anion exchange resin (Amberlite IRA-400, Cl^- form), and the same solvent mixture was used to elute the alkaloid chlorides. The solution containing the alkaloid chlorides was evaporated until removal of acetone and methanol was complete. The remaining aqueous solution was basified with 10% ammonia and extracted (5 x 1 liter) with chloroform. The dried (Na_2SO_4) chloroform phase was evaporated to dryness (residue A) (19 g), while the aqueous phase was freeze dried (residue B) (23 g).

ISOLATION AND CHARACTERIZATION OF THE ALKALOIDS.—Residue A (8 g) was dissolved in chloroform, chromatographed over silica gel, and eluted first with chloroform and subsequently with increasing concentrations of methanol in chloroform, up to 30% methanol. Twenty ml fractions were collected and were grouped according to the results of the tlc check. From different groups, five of the alkaloids were obtained pure with the aid of preparative tlc.

PHYSICAL DATA OF THE ISOLATED ALKALOIDS

AKAGERINE (**1**).—Crystallized from ethanol-diethyl ether (18 mg), mp 181–184° (dec.) (lit (4). mp 178–182° (dec.)); uv λ max 229, 278, 283 and 296 nm (log ε 4.41, 3.78, 3.78 and 3.72); ir ν max 3340, 3040, 2920, 2840, 2700, 1680, 1630, 1465, 1450, 1375, 1310, 1300, 1210, 1105, 1095, 930, 895 and 740 cm^{-1}; ms *m/z* (%): 324 (M^-, 20), 309 (5), 306 (11), 281 (12), 214 (9), 213 (12), 198 (13), 186 (15), 185 (100), 184 (21), 183 (16), 180 (10), 171 (40), 156 (13), 144 (10) and 143 (10); H^1-nmr 9.24 (1H, *s*, H-21), 7.43–6.84 (4H, *m*, H-9, H-10, H-11, H-12), 6.43 (1H, *q*, *J*=7 Hz, H-19), 6.11 (1H, *m*, H-17), 2.41 (3H, *s*, N-CH_3) and 2.03 (3H, *d*, J=7 Hz, H-18). The material isolated was chromatographically and spectrally identical with an authentic sample of akagerine (3–5). The R_f-values for akagerine in the tlc systems A, B and C were 0.39, 0.16 and 0.57.

17-*O*-METHYLAKAGERINE (**2**).—Crystallized from aqueous ethanol, 17-*O*-methyl-akagerine was obtained as pale yellow prisms, (81 mg) mp 183–185° (dec.) (lit (4). mp 187–189°); uv λ max 229, 278, 282.5, 292 and 306 nm (log ε 4.47, 3.78, 3.78, and 3.67); ir ν max 2940, 2840, 2790, 1680, 1640, 1465, 1380, 1310, 1270, 1120, 1090, 1060, 870 and 740 cm^{-1}; ms *m/z* (%): 338 (M^+, 60) 337 (20), 323 (17), 307 (20), 295 (17), 277 (25), 264 (13), 263 (10), 237 (17), 236 (40), 235 (21), 222 (14), 220 (15), 214 (16), 213 (100), 212 (12), 185 (34), 184 (39), 183 (32), 180 (20), 168 (11), 156 (14) and 144 (10); ^{1}H-nmr δ 9.29 (1H, *s*, H-21), 7.56–6.96 (4H, *m*, H-9, H-10, H-11, H-12), 6.50 (1H, *q*, J=7 Hz, H-19), 5.65 (1H, *s*, H-17), 3.06 (3H, *s*, O-CH_3), 2.53 (3H, *s*, N-CH_3), and 2.03 (3H, *d*, J=7 Hz, H-18). The isolate was identical with authentic 17-*O*-methyl-akagerine (4) by comparison of tlc, mmp, uv, ir, ms, and ^{1}H-nmr. The R_f-values in the tlc systems A, B and C were 0.63, 0.46 and 0.75.

10-HYDROXY-21-*O*-METHYL-KRIBINE (**3a**).—The compound was purified by preparative tlc using ether-ethanol-diethylamine (90:3:7) and elution with chloroform-methanol (12 mg), mp 145–148° (dec.); uv λ max 215, 269, 312 and 322 (sh) nm (log ε 4.51, 4.62, 4.08, 4.03); λ max (addition of KOH) 234, 284, 330 nm (log ε 4.50, 4.52 and 4.15); ir ν max 3360, 2940, 2800, 1680, 1630, 1610, 1480, 1320, 1230, 1180, 1130, 1060, 990, 940, 855, 800 and 745 cm^{-1}; ms *m/z* (%): 354 (M^+, 100), 353 (44), 339 (12), 323 (23), 322 (11), 311 (25), 296 (18), 295 (25), 293 (18), 281 (16), 279 (20), 253 (33), 252 (96), 251 (77), 250 (13), 238 (25), 237 (18), 236 (23), 226 (21), 222 (13), 210 (13), 209 (13), 200 (15), 199 (17), 196 (12), 185 (36), 126 (14) and 107 (23). Analysis by high resolution ms.

---

[3]The ir spectra were obtained on a Jasco-IRA-I-spectrophotometer in KBr tablets, while the uv spectra were recorded in ethanol solution on a Shimadzu MPS-5000 uv-vis spectrophotometer. Mass spectral analyses were carried out with an LKB 9000 instrument at 70 eV with direct inlet. The ^{1}H-nmr spectra were obtained on a Jeol 100 MHz instrument and/or a Varian 100 MHz instrument, in $CDCl_3$ with TMS as internal standard. The ^{13}C-nmr spectra were recorded on the Varian 100 MHz instrument. Melting points were determined with a Leitz mikroskopheitztisch 350.

Thin-layer chromatography was carried out on either precoated silica gel 60 plates (0.25 mm silica F_{254}, E. Merck) or on a 0.50 mm layer of silica gel GF_{254} (type 60, E. Merck) spread on 20 x 20 cm glass plates. Silica gel 60 (70–230 mesh, E. Merck) was used for column chromatography. Tlc systems used:

A. Cyclohexane:chloroform:diethylamine (5:4:1)
B. Chloroform:methanol (9:1)
C. Diethylether:ethanol:diethylamine (90:3:7)

Found: M⁻354.1948. $C_{21}H_{26}N_2O_3$ requires: 354.1943. ^{1}H-nmr[4] δ 7.15 (1H, *d*, J=9 Hz, H-12), 6.87 (1H, *d*, J=2 Hz, H-9), 6.75 (1H, *dd*, J=9 Hz, 2 Hz, H-11), 5.14 (1H, broad *s*, exchangeable with D_2O), 4.86 (1H, *m*, H-17), 4.54 (1H, *q*, J=6 Hz, H-19), 3.44 (3H, *s*, O-CH_3), 2.52 (3H, *s*, N-CH_3), and 1.46 (3H, *d*, J=6 Hz). The R_f-values in the tlc systems A, B and C were 0.20, 0.31 and 0.34.

10-HYDROXY-EPI-21-*O*-METHYL-KRIBINE (**3b**).—On purification by the procedure adopted for **3a**,10-hydroxy-epi-21-*O*-methyl-kribine was obtained as an amorphous powder (13.9 mg), mp 176–180° (dec.); uv λ 216, 270, 312 and 322 (sh) nm (log ε 4.56, 4.60, 4.08 and 4.01); λ max (addition of KOH) 229, 286, 329 nm (log ε 4.39, 4.21 and 4.13); ir ν max 3400, 2930, 2860, 1680, 1630, 1610, 1480, 1460, 1390, 1310, 1230, 1190, 1180, 1090, 1070, 870, 810, and 750 cm^{-1}; ms *m/z* (%): 354 (M^+, 77), 353 (37), 323 (20), 322 (40), 311 (18), 295 (30), 293 (25), 280 (15), 279 (40), 265 (20), 254 (33), 252 (100), 251 (54), 239 (18), 237 (23), 236 (32), 226 (26), 223 (16), 210 (17), 209 (17), 200 (21), 199 (20), and 140 (16). Analysis by high resolution ms. Found: M^+354.1940. $C_{21}H_{16}H_2O_3$ requires: 354.1943. ^{1}H-nmr[4] δ 7.15 (1H, *d*, J=9 Hz, H-12), 6.85 (1H, *d*, J=2 Hz, H-9), 6.75 (1H, *dd*, J=9 Hz, 2 Hz, H-11), 5.13 (1H, broad *s*, exchangeable with D_2O), 4.67 (1H, *m*, H-17) 4.11 (1H, *q*, J=6 Hz, H-19), 3.56 (3H, *s*, O-CH_3), 2.52 (3H, *s*, H-CH_3) and 1.52 (3H, *d*, J=6 Hz); ^{13}C-nmr: C-2 137.0[1], C-3 57.3, C-5 52.8, C-6 20.2, C-7 108.3, C-8 127.3, C-9 103.6, C-10 151.7, C-11 109.9, C-12 112.1, C-13 131.6[1], C-14 39.5, C-15 36.4, C-16 34.9, C-17 72.8, C-18 17.6, C-19 117.3, C-20, 122.3, C-21 102.5, N-CH_3 42.1, C_{21}-O-CH_3 56.2 ([1]signals may be reversed). The R_f-values in the solvent systems A, B and C were 0.20, 0.24 and 0.33.

10-HYDROXY-17-*O*-METHYL-AKAGERINE (**4**).—Purified by preparative tlc using ether-ethanol-diethylamine (90:3:7), 10-hydroxy-17-*O*-methyl-akagerine was obtained as white crystalline solid, (30.5 mg); mp 135–138° (dec.); uv λ max 232, 279, 301 and 305 nm (log ε 4.52, 4.20, 3.86 and 3.70); λ max (addition of KOH) 239, 275 and 328 nm (log ε 4.68, 4.18 and 4.13); ir ν max 3280, 2940, 2850, 2800, 1680, 1635, 1595, 1475, 1455, 1380, 1360, 1220, 1160, 1120, 1095, 1060, 920, 880, 840, and 800 cm^{-1}; ms *m/z* (%) 354 (M^-, 26), 339 (12), 323 (31), 322 (95), 321 (30), 307 (16), 294 (18), 293 (47), 279 (35), 265 (18), 253 (20), 251 (21), 239 (38), 238 (18), 229 (83), 223 (23), 214 (21), 213 (16), 209 (16), 201 (42), 200 (100), 199 (49), 197 (24), 196 (57), 185 (17), 184 (16), 183 (19), 172 (18), and 107 (18). Analysis by high resolution ms. Found: M^- 354.1939. $C_{21}H_{26}N_2O_3$ requires: 354.1943. ^{1}H-nmr δ 9.25 (1H, *s*, H-21), 7.20 (1H, *d*, J=9 Hz, H-12), 6.90 (1H, *d*, J=2 Hz, H-9), 6.75 (1H, *dd*, J=9 Hz, 2 Hz, H-11), 6.49 (1H, *q*, J=7 Hz, H-19), 5.53 (1H, broad *s*, H-17), 5.25 (1H, *s*, exchangeable with D_2O) 3.03 (3H, *s*, OCH_3), 2.54 (3H, *s*, N-CH_3), 1.98 (3H, *d*, J=7 Hz). The R_f-values in the tlc systems A, B and C were 0.20, 0.24 and 0.33.

PHARMACOLOGICAL ACTIVITY

The alkaloids tested were weighed as bases; an equivalent amount of citric acid was added, and the mixture was dissolved in 0.9% w/v NaCl. Female mice of the NMRI strain, weighing 18–20 g were injected intraperitoneally with the solutions of the alkaloids in 2–3 doses. The mice were observed for at least one hour for the occurrence of clonic and tonic convulsions (13). The values for CD_{50} (convulsive dose for 50% of the animals) and LD_{50} are given as base in mg/kg of body weight, and were calculated by probit analysis (14).

ACKNOWLEDGMENTS

We thank Mr. D. Carter, Mass Spectrometry Service, The School of Pharmacy, University of London, and Mr. P. Jahnke, Biomedical Center, Uppsala University, for the mass spectra. Dr. Toshi Nishida, Tobacco Company, Stockholm, Mr. McDonough, Chelsea College, University of London, and Mr. G. Everit, National Board of Health and Welfare, Division of Drugs, Uppsala, Sweden, are gratefully acknowledged for obtaining and discussion of the nmr-spectra. Our gratitude is also extended to Drs. L. Bohlin, R. Verpoorte, J. G. Bruhn, and Prof. F. Sandberg for useful discussions. One of the authors (W. R.) wishes to thank The Swedish Academy of Pharmaceutical Sciences for financial support during her stay at Chelsea College, London, October-November 1977, while A. A. O. expresses his appreciation to the International Seminar in Physics and Chemistry, University of Uppsala, for the award of a research fellowship.

Received 18 April 1979.

LITERATURE CITED

1. M. Geevaratne, W. Rolfsen and L. Bohlin, *Acta Pharm. Suec.*, **14**, 43 (1977).
2. A. Petitjean, P. Rasoanaivo and J. M. Razafintsalama, *Phytochemistry*, **16**, 154 (1977).
3. L. Angenot, D. Dideberg and L. Dupont, *Tetrahedron Lett.*, **16**, 1357 (1975).
4. R. Verpoorte, A. B. Svendsen and F. Sandberg, *Acta Pharm. Suec.*, **12**, 455 (1975).
5. W. Rolfsen, L. Bohlin, S. K. Yeboah, M. Geevaratne and R. Verpoorte, *Planta Med.*, **34**, 264 (1978).

[4]The multiplet of H-21, present in 21-*O*-methylkribine and epi-21-*O*-methylkribine, is presumably hidden in the region of the aromatic protons in 3a's and 3b's ^{1}H-nmr spectra.

6. L. Akhter, R. T. Brown and D. Moorcroft, *Tetrahedron Lett.*, **43**, 4137 (1978).
7. G. Lewin, N. Kunesch, A. Cave, T. Sevenet and J. Poisson, *Phytochemistry*, **14**, 2067 (1975).
8. A. Banerji and M. Chakrabarty, *Phytochemistry*, **13**, 2309 (1974).
9. L. Angenot, C. Coune and M. Tits, *J. Pharm. Belg.*, **33**(1), 11 (1978).
10. H. Rosler, H. Fram and R. N. Blomster, *Lloydia*, **41**(4), 383 (1978).
11. Y. Ahmad and P. W. Le Quesne, *J. Chem. Soc. Chem. Commun.*, 1970, 538.
12. F. W. Wehrli and T. Wirthlin, "Interpretation of carbon-13 NMR spectra", Heyden, 1976. p. 47.
13. F. Sandberg, R. Verpoorte and A. Cronlund, *Acta Pharm. Suec.*, **8**, 341 (1971).
14. D. J. Finney, "Probit analysis", Cambridge University Press, Cambridge, 1947, p. 199.
15. Pure Appl. Chem., **50**, 65 (1978). Recommendations for symbolism and nomenclature of mass spectrometry.