Mass Spectral Analysis of Glucuronides from Sympathomimetic Hydroxyphenylalkylaminoethanols

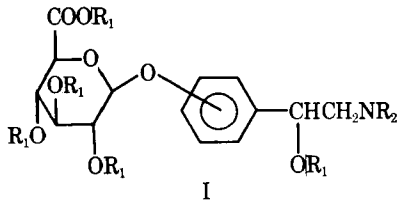
KARL-HEINZ POOK ˣ, KARL-LUDWIG ROMINGER, and DIETRICH ARNDTS

Abstract □ A mass spectral method is described for the structure determination of glucuronic acid conjugates of hydroxyphenylalkylaminoethanol-type drugs. Trimethylsilylation and application of the GLC–mass spectral technique yield mass spectra with sufficient information for the identification of all structural subunits.

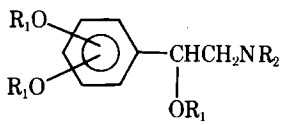
Keyphrases □ Hydroxyphenylalkylaminoethanols—mass spectral analysis of glucuronides □ Glucuronides—hydroxyphenylalkylaminoethanol-type drugs, mass spectral analysis □ Mass spectrometry—analysis, glucuronides from sympathomimetic hydroxyphenylalkylaminoethanols

The identification of glucuronides obviously depends on observations revealing that the role of conjugates is not exclusively one of detoxification (1). Some reports on the GLC properties of urinary steroid glucuronides presented routine separation methods (2, 3). The application of the GLC–mass spectral technique to some glucuronides derived from phenolic and steroidal aglycons showed that this method, in principle, is suitable for direct identification (4). The glucuronides derived from several barbitals (5) and aromatic acids as well as substituted phenols (6) were studied by similar techniques.

Glucuronides (I, R_1 = H, R_2 = alkyl), produced by the primary route of metabolism of the hydroxyphenylalkylaminoethanol-type drugs (II, R_1 = H, R_2 = alkyl), were investigated. The ratio of glucuronized and free drug concentrations determined in blood plasma was up to 9:1. Since a general GLC–mass spectral study (7) on catecholamine-type derivatives (II, R = trimethylsilyl or trifluoroacetyl) yielded mass spectral data for metabolite identification, the derivatizing procedure for trimethylsilyl derivatives and the inlet system conditions were modified to measure the glucuronides (I). To support the mass spectral data obtained from 10 conjugates, deuterated derivatives and one model compound were studied [Table I and Structures V–XIV, R = $Si(CH_3)_3$].



I



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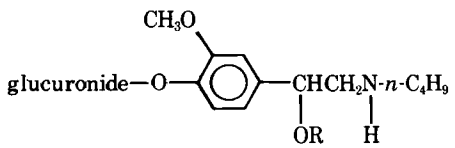
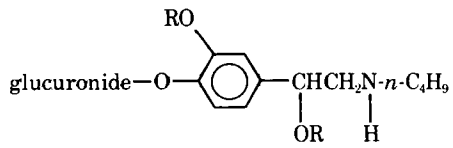
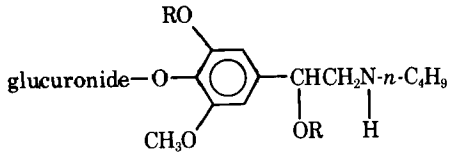


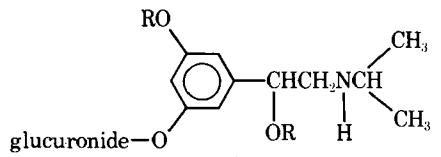
V: M^+775



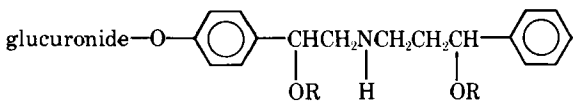
VI: M^+833



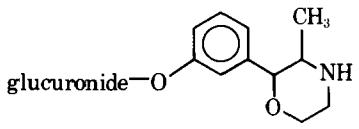
VII: M^+863



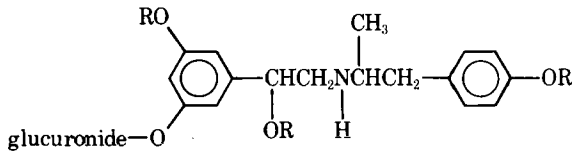
VIII: M^+819



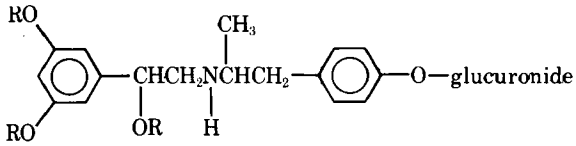
IX: M^+895



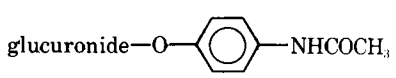
X: M^+657



XI: M^+983



XII: M^+983



XIII: M^+615

Table I—Mass Spectra Data: *O*-Trimethylsilylglucuronides (*m/e*, Relative Intensity)

Compound	R_1	R_2	M+	M−15	M−15−90(−90)	M−α −90	M−α −465[a]	α	M−Aglycon		Metastable Transitions
III[c]	$Si(CH_3)_3$	n-C_4H_9	745 (16.6)	730 (10.0)	640 (3.8) 550 (0.5)	659 (6.6)	569 (3.6)	267 (88) 268 (22) 195 (90) 196 (37)	86 (95)	375 (100) (c)[b]	465 (2.4)	583.0 561.0 302.5	745 → 659 730 → 640 465 → 375
IIIa	$Si(CD_3)_3$	n-C_4H_9	790 (15.5)	773 (8)	673 (3)	704 (5.8)	605 (3.3)	285 (80) 286 (19) 204 (88) 205 (17)	86 (90)	402 (100) (c')[b]	501 (2)	627.5 322.8	790 → 704 501 → 402
IV[d]	$Si(CH_3)_3$	C_2H_5	717 (18)	702 (10)	612 (1.8)	659 (1.8)	569 (1.3)	267 (23) 268 (93) 195 (78) 196 (65)	58 (90)	375 (100) (c)[b]	465 (3)	606.0 302.5 196.3	717 → 659 465 ↑ 375 717 ↑ 375
IVa	$Si(CD_3)_3$	C_2H_5	762 (15)	744 (7.9)	645 (1.6)	704 (1.6)	605 (1.2)	285 (20) 286 (81) 204 (69) 205 (49)	58 (92)	402 (100) (c')[b]	501 (2.8)	651.0 322.8 212.0	762 → 704 501 ↑ 402 762 ↑ 402
								333 (30) 257 (34) 217 (93) 204 (69)	(c)[b]	360 (29) 275 (30) 235 (90) 204 (65)	(c')[b]		

[a] (M − α) − 465 + $(CH_3)_3Si/H$. [b] Indicates paths *c* and *c'* degradation of 375/402. [c] Glucuronide–O at 4-position; Vasculat (Bamethane), C. H. Boehringer Sohn, West Germany. [d] Glucuronide–O at 3-position; Effortil (Etilefrine), C. H. Boehringer Sohn, West Germany.

EXPERIMENTAL

Isolation and Preparation of Materials—The glucuronides were isolated from rat urine and purified using ion-exchange chromatography and TLC. Column materials and solvents for repeated purification steps were adapted to the various types of conjugates[1].

Additional TLC purification was necessary to isolate IX and X; 20 × 20-cm glass plates precoated with silica gel[2] and 1-butanol–acetic acid–water (50:10:40 v/v) were used. Elution time was up to 72 hr, if required. The fractions eluted were concentrated to dryness by lyophilization. The concentration ratio of radiolabeled drugs and their glucuronides in blood plasma was determined following their chromatographic separation and enzymatic hydrolysis.

Derivatizing Procedure—One milligram of glucuronide was dissolved in 40 μl of pyridine, analytical grade, and 40 μl of *N,O*-bis(trimethylsilyl)acetamide – *N,O*-bis(trimethylsilyl)trifluoroacetamide (50:50) was added. The sample was warmed slightly and kept in a closed vial at room temperature for 5 hr. Glucuronides containing larger substituents, *i.e.*, VIII, required 10–15 hr for derivatization.

GLC–Mass Spectral Conditions—One-microliter volumes were injected, using a gas chromatograph[3] equipped with a 1.50-m × 2-mm i.d. column packed with 1% OV-17 (or 3% OV-17) and operating at a helium flow rate of 25 ml/min. The temperature was programmed between 180 and 330° at 6°/min. The molecular separator[4], kept at 250°, was connected to a mass spectrometer[5] with the source at 220°, the electron energy at 70 ev, and the acceleration voltage at 3 kv. Mass spectra were obtaining by scanning 100 mass units in 2 sec and recorded on an electrostatic recorder[6].

Figure 1 shows typical elution profiles monitored by the total ion current trace, which was also used as a signal for the scan start of the mass spectrometer. In some cases (Fig. 1*a*), a peak at a shorter retention time (peak 1) was present; it was identified as the *O*-trimethylsilyl derivative of the corresponding aglycon. This peak was due to partial cleavage of the glucuronide acetal bond during derivatization. Isomeric glucuronides (Fig. 1*b*) with higher molecular weights were separated satisfactorily.

RESULTS AND DISCUSSION

In molecules such as I, the glucuronic acid moiety and the alkylaminoethanol side chain are linked together by the rather stable benzene ring. Therefore, both structural subunits give rise to a selective and characteristic fragmentation, which is easily extractable from the total pattern and may be correlated with the sugar acid and the aglycon partial structures, respectively. The data from two typical *O*-trimethylsilylglucuronides (III and IV) and their deuterated analogs (III*a* and IV*a*) are summarized in Table I.

The mass spectrum resulting from III (Fig. 2) represents the general pattern consistently observed in all spectra from homologous glucuronides IV–VIII[7]. As shown in Scheme I, three particular sequences can be deduced. The molecular ion, generally appearing with a reasonable intensity, runs through the M − 15 − 90 − 90 sequence (Path *a*), yielding *m/e* 730, 640, and 550. Loss of trimethylsilyl hydroxide from the 1,2-position in the aglycon side chain may contribute to these ions (7, 8). Numerous injections performed in this work demonstrated that there was no need to rely upon a "molecular ion set" as mentioned previously (4).

For unequivocal identification of the aglycon side chain, the *O*-aryl C-1 portion and the sugar acid moiety key fragments formed by paths *b* and *c* are used. Upon C-1–C-2 bond cleavage, the α-fragment, *m/e* 86, and the M − α ion, *m/e* 659, occur. The latter, still containing the sugar acid ring, yields (M − α) − 90, *m/e* 569, with weaker intensity. The main degradation induced by the acetal bond cleavage, as indi-

[1] For III and V–VII, the columns were Zerolit M-IP (SRA-153) and Sephadex QAE-A25 in acetate form; the elution solvent was linear gradient acetic acid–water. For IV, the columns were Amberlite CG-50-I in ammonium form and Zerolit in acetate form; the elution solvent was hydrochloric acid. For VIII (Alupent) and IX, the columns were Amberlite, Zerolit, and Sephadex; the elution solvent was the same as for III. For X, the columns were Amberlite and Sephadex; the elution solvent was the same as for III. For XI (Berotec)–XIII, the columns were Zerolit and Sephadex; the elution solvent was the same as for III.

[2] F-254, Merck 5715/0025.

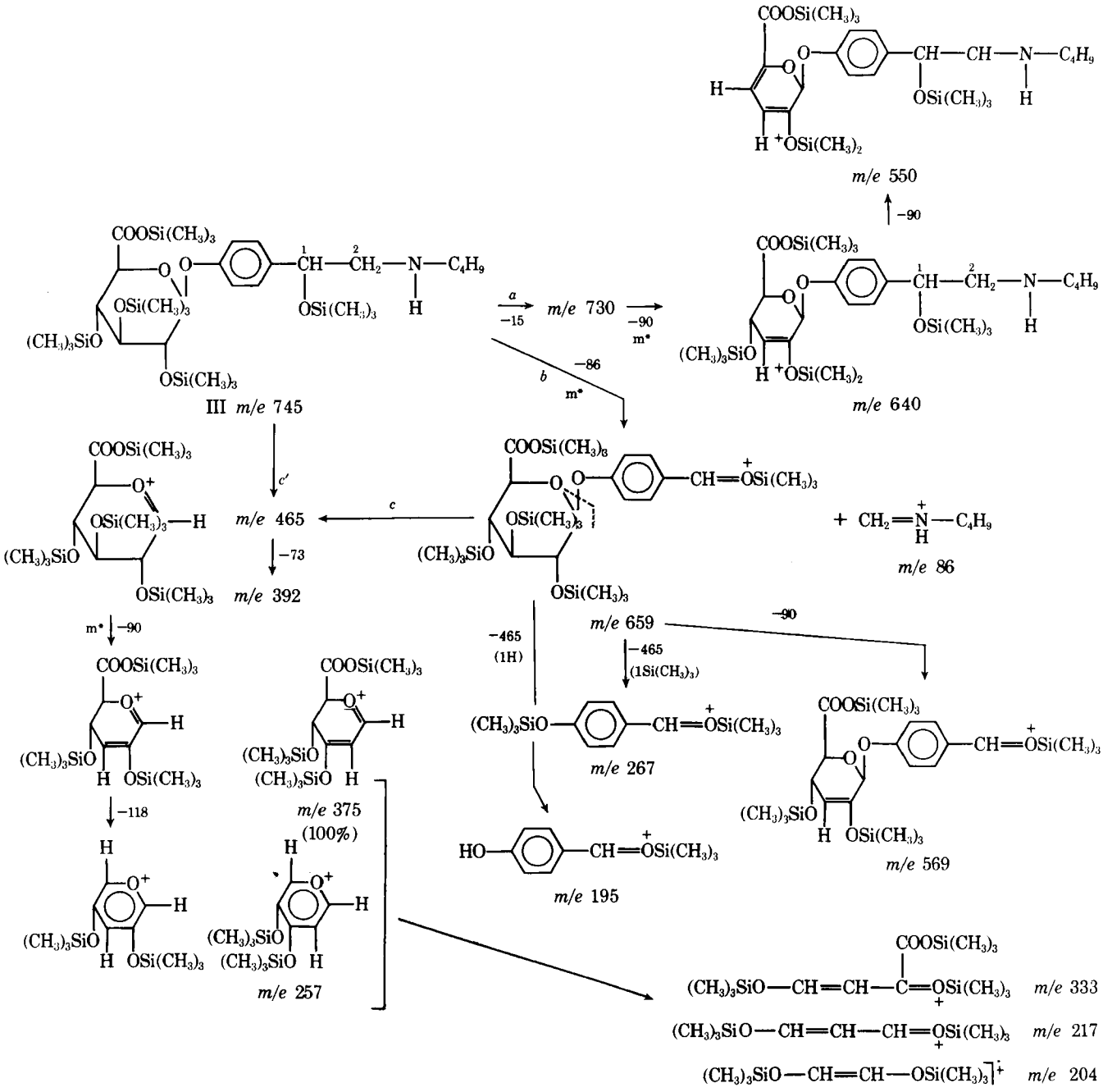
[3] Varian Aerograph 700.

[4] Watson-Biemann.

[5] Varian-Mat CH-7.

[6] Varian-Statos recorder.

[7] Modifications concerning IV, IV*a*, and VIII are mentioned later.



Scheme I

cated, leads to *m/e* 465 and, by stabilization of the residual fragment by trimethylsilyl or proton addition, to *m/e* 267 (7, 9) and 195.

The oxonium ion, *m/e* 465, could also be formed by path *c'* by direct acetal bond cleavage from the molecular ion; the M − 465 residue then undergoes α-cleavage and stabilization to *m/e* 267 and 195 as described. The metastable ion found for the M − α transition, however, indicates that path *b* should be predominant[8].

By multiple loss of trimethylsilyl hydroxide and ring fragmentation of *m/e* 465, the sequence of ions *m/e* 375 (normally the base peak), 333, 257, 217, and 204 is produced selectively, indicating the presence of the silylated glucuronic acid ring. Similar fragmentation was observed when carbohydrate derivatives were examined (9).

As shown in Fig. 2, the sequence resulting in paths *b* and *c* is predominant in the spectrum.

The data from V, VI, and VII clearly correspond to those of III; the appropriate ions are shifted 30, 88, and 119 mass units, respectively. The deuterium-labeled derivative (III*a*) also had the correct mass shifts at the key ions, as listed in Table I.

Glucuronides derived from aglycons with 3′- or 3′,5′-hydroxylation in the benzene ring such as IV, IV*a* (Table I), and VIII show an additional significant feature concerning the C-1–C-2 bond cleavage: the characteristic benzyl fragments (IV and IV*a*) *m/e* 267, 285 and 195, 204 are accompanied by intense peaks *m/e* 268, 286 and 196, 205, respectively (Fig. 3). For example, the ratio of intensity of *m/e* 267/268 as found for III (approximately 80:20) is completely reversed in the spectrum of IV. This result must be due to a markedly favored McLafferty rearrangement (10) for the loss of the C-2 NR portion (Scheme II) in the case of 3′- or 3′,5′-aryl substitution (the latter leading to *m/e* 356/355 due to the second trimethylsilyloxy group on the aryl ring).

Two structural variations concerning the aglycon side chain are illustrated by IX and X. Compound X obviously displays little fragmentation from the morpholine ring, except losing the methyl group and thus probably contributing to the *m/e* 642 ions (Fig. 4). Ions usually found in phenmetrazine (2-phenyl-3-methylmorpholine) mass spectra are completely absent (11). Consequently, the M − 15 frag-

[8] Several transitions are confirmed by metastable peaks, as listed in Table I.

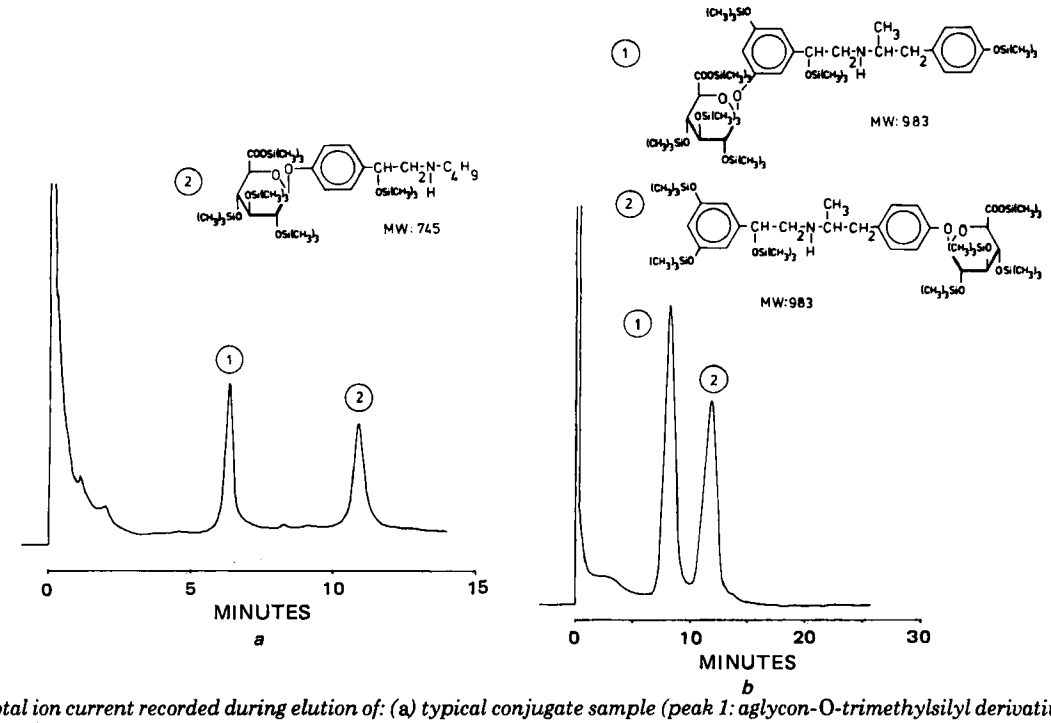


Figure 1—*Total ion current recorded during elution of: (a) typical conjugate sample (peak 1: aglycon-O-trimethylsilyl derivative, programmed at 180–310°, 10°/min); and (b) two isomeric glucuronide derivatives, XI (peak 1) and XII (peak 2).*

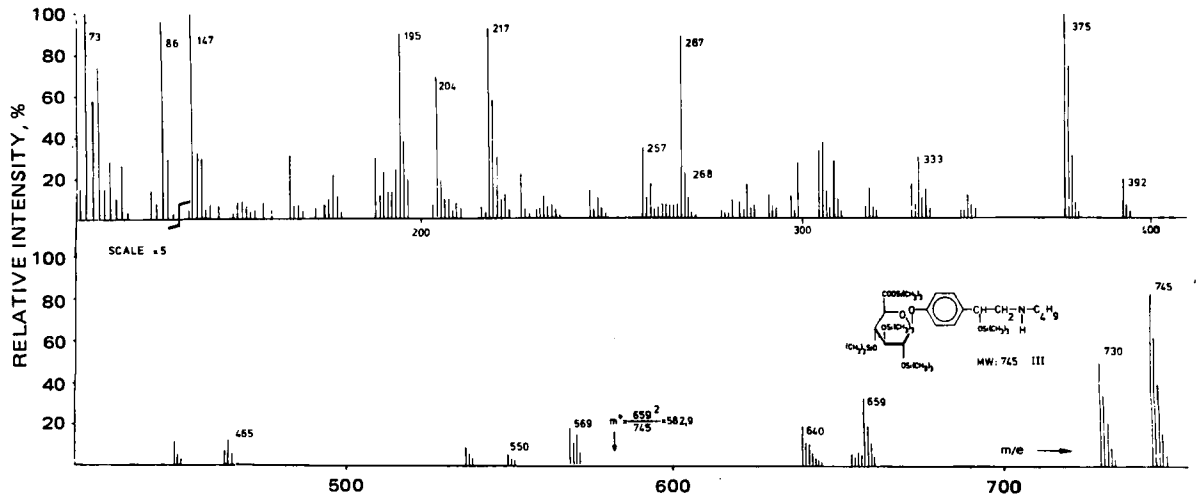


Figure 2—*Mass spectrum of glucuronide derivative III.*

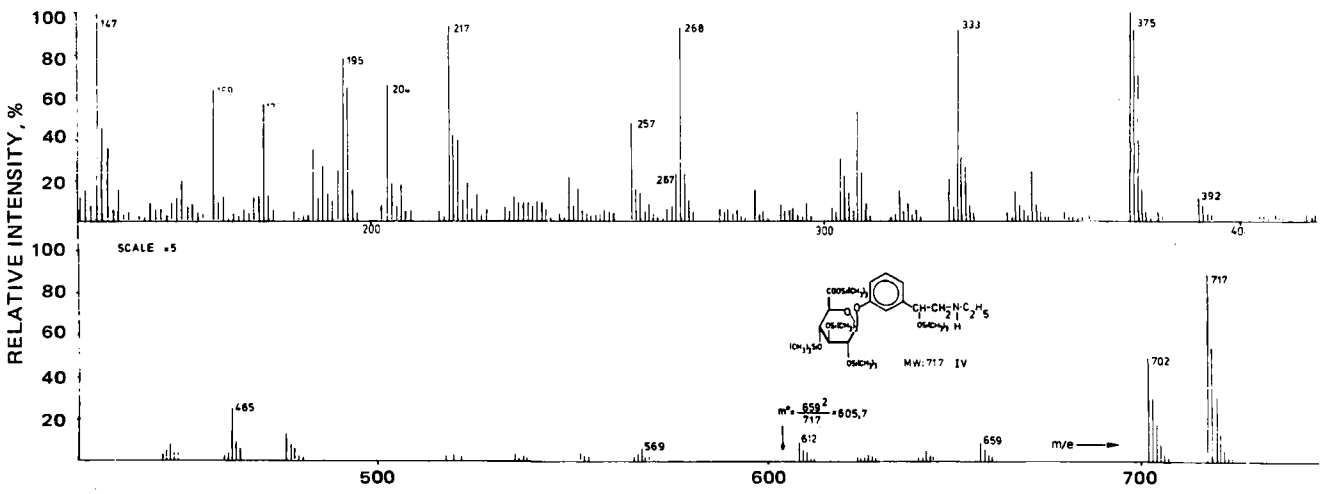


Figure 3—*Mass spectrum of glucuronide derivative IV.*

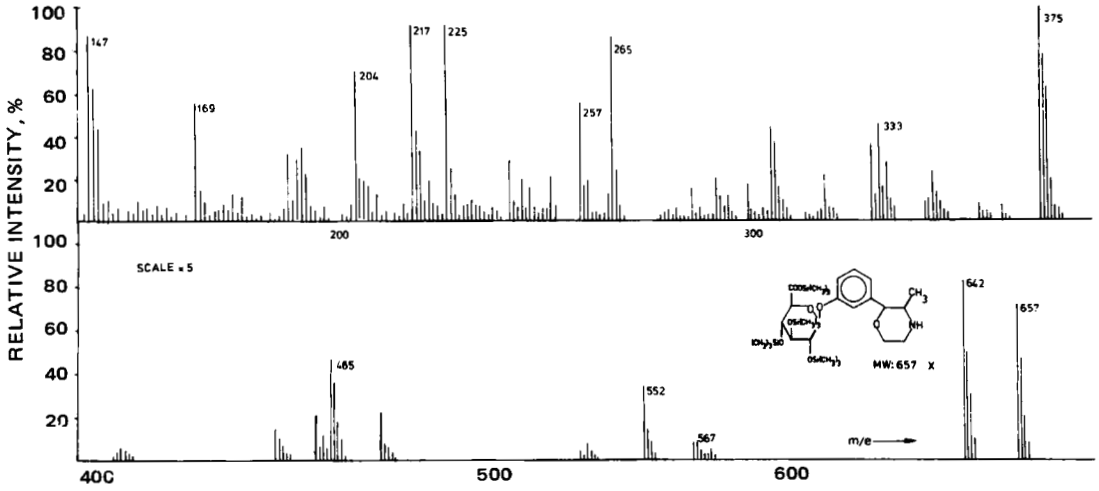


Figure 4—*Mass spectrum of glucuronide derivative X.*

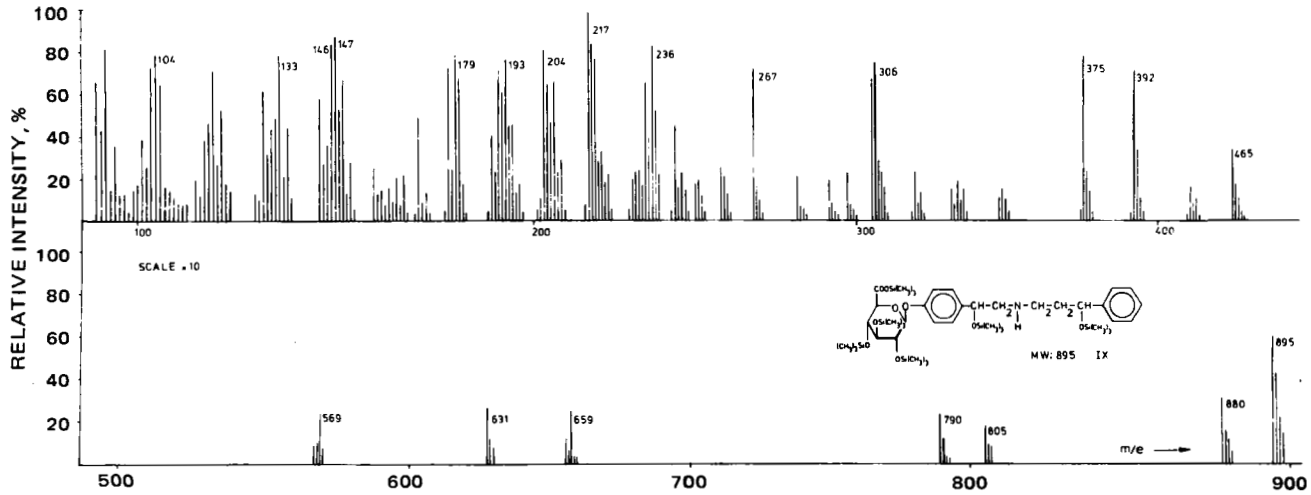
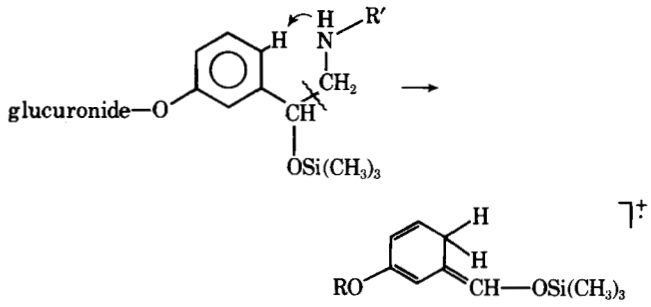


Figure 5—*Mass spectrum of glucuronide derivative IX.*

ment, *m/e* 642 (instead of the M − 15 − α analog), undergoes acetal cleavage followed by trimethylsilyl transfer, as described, to form *m/e* 265; the fragment resulting from proton stabilization, *m/e* 179, is not present in reasonable intensity. This type of spectrum also is observed if glucuronides such as XIII are measured.

Attachment of a phenylhydroxypropyl group at the nitrogen, as is present in IX (Fig. 5 and Scheme III), naturally yields an increased fragmentation within the aglycon portion due to multiple α-cleavage as the ions *m/e* 236, 193, 179, 146, and 104 reveal. The *m/e* 465 degradation sequence is not markedly suppressed in intensity but modified, *m/e* 217 forms the base peak instead of *m/e* 375, and *m/e* 392 (Scheme I and Fig. 2) appears much more intense than in normal glucuronide spectra.

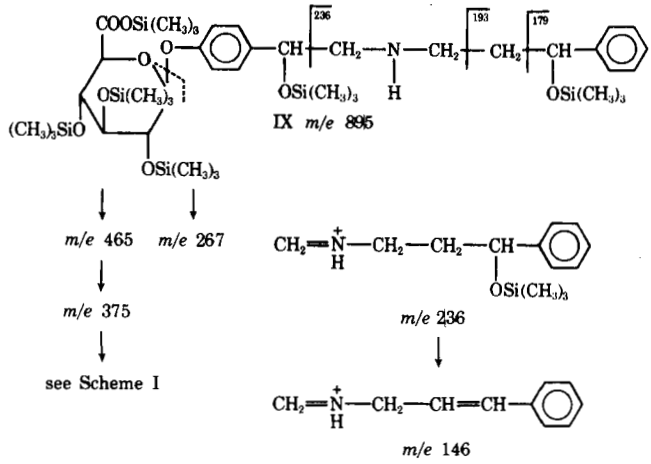


i.e. from IV: R = $Si(CH_3)_3$, *m/e* 268
R = H, *m/e* 196
from IV*a*: R = $Si(CD_3)_3$, *m/e* 286
R = H, *m/e* 205

Scheme II

In a special conjugate sample, originating from a new bronchodilator, a mixture of two glucuronides, XI and XII, could be determined after GLC separation of the trimethylsilyl derivatives (mol. wt. 983). Peak 1 (Fig. 1*b*) was identified as the 3′-conjugate species (Fig. 6). Key ions detecting the arylalkylamino side chain are generated by the first and second α-cleavage, yielding *m/e* 236 and 179, and, in addition, *m/e* 207 from *m/e* 236 by loss of 29.

The M − 179 = *m/e* 804 represents the base peak; M − 236 = *m/e* 747 incorporates the whole glucuronide–*O*-aryl–C-1 moiety. As already described, the typical stabilization steps following the acetal cleavage form *m/e* 283 and 355 (Scheme I, 195, 267). Intense ions at



Scheme III

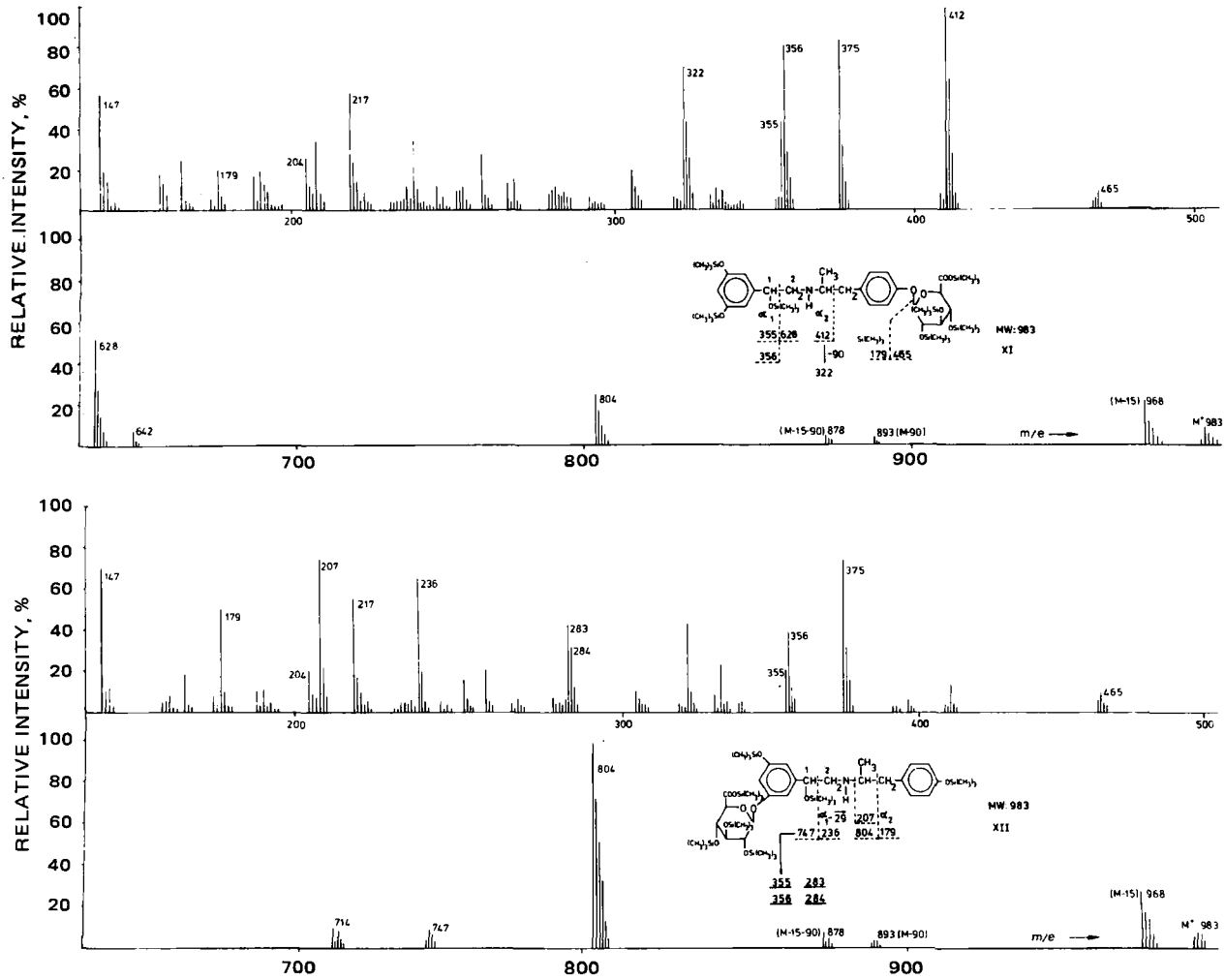


Figure 6—*Mass spectra of the isomeric glucuronide derivatives XI and XII.*

284 and 356 mass units indicate again a predominance of the McLafferty mechanism for the loss of the C-2 NR portion (Scheme II).

The mass spectrum resulting from peak 2 (Figs. 1*b* and 6) demonstrates a different pattern, since the sugar acid portion is attached at the 4′-hydroxyl function in the phenylpropyl moiety. The characteristic ions formed upon double α-cleavage are *m/e* 628 and 412; the latter loses trimethylsilyl hydroxide to give *m/e* 322. The 355/356 ions are present in approximately the same intensity ratio as is found with XI. The M − 412 (α_2) = *m/e* 517 is not detected, obviously due to rapid acetal cleavage/trimethylsilyl transfer forming *m/e* 179. Unequivocal structural differentiation of these more complicated isomeric conjugates is thus performed by interpretation of rather few typical fragments.

CONCLUSION

The method is easy to use for routine identification of 100-μg samples of glucuronides. Work is in progress to improve the mass spectrometric sensitivity and to modify the method for quantitation.

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