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USE OF THERMOSPRAY LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY FOR CHARACTERIZATION OF REACTIVE METABOLITES OF 3′-HYDROXYACETANILIDE, A NON-HEPATOTOXIC REGIOISOMER OF ACETAMINOPHEN

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SUMMARY

3′-Hydroxyacetanilide (AMAP) is a non-hepatotoxic regioisomer of acetaminophen that nonetheless does form reactive metabolites that are trapped as glutathione thioether adducts. These reactive intermediates are, 4-acetamido-*o*-benzoquinone, 2-acetamido-*p*-benzoquinone and N-acetyl-3-methoxy-*p*-benzoquinone. Thermospray liquid chromatography–mass spectrometry (TSP LC–MS) was used to characterize products of reactions of these reactive compounds with cysteine or N-acetylcysteine. The TSP spectra of the *mono-* and *bis*-thioether adducts showed protonated molecular ions and characteristic fragmentation patterns. The chromatographic resolution together with the MS selectivity allowed for unequivocal identification of these conjugates in the urine of mice treated with AMAP.

INTRODUCTION

Thermospray liquid chromatography–mass spectrometry (TSP LC–MS) is one of several techniques developed in the last few years to overcome the inherent difficulty in interfacing the eluent of an high-performance liquid chromatograph to the source of a mass spectrometer. Since it first appeared[1] and for the last few years TSP LC–MS has proven to be a valuable technique applicable to drug metabolism studies and in routine pharmaceutical and environmental analysis[2–6]. The technique can handle normal and reversed-phase solvent systems at flow-rates of 0.5–2 ml/min and the ionization is soft, which allows for the analysis of polar, non-volatile thermally labile chemicals present in plasma or urine or other biological matrices.

Glutathione (GSH) adducts of xenobiotics and their metabolic breakdown products, namely the cysteine and N-acetylcysteine conjugates, are very polar and thermally labile compounds and therefore require softer modes of ionization such as desorption chemical ionization (DCI)[7], field desorption (FD)[8] and fast atom bombardment (FAB)[9]. More recently[10] several model compounds of this type have been studied by thermospray ionization mass spectrometry. In addition, Conchillo *et al.*[11] reported that under thermospray conditions sensitivities for GSH adducts were poor and gave unsatisfactory spectra[11]. Instead the analysis of the methyl esters of the corresponding cysteine or N-acetylcysteine derivatives afforded better spectra and

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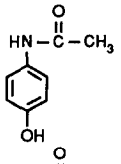
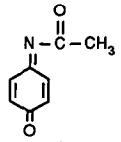
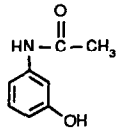
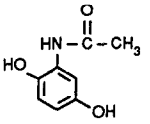
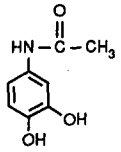
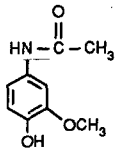
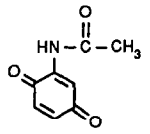
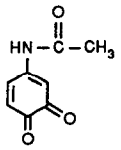
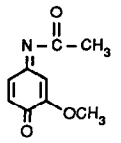
TABLE I
STRUCTURE ABBREVIATIONS AND CHEMICAL NAMES OF COMPOUNDS MENTIONED IN THE TEXT

Structure	*Abbreviation*	*Chemical name*
	APAP	4′-Hydroxyacetanilide
	NAPQI	N-Acetyl-*p*-benzoquinone imine
	AMAP	3′-Hydroxyacetanilide
	2-AcHQ	2-Acetamidohydroquinone
	3-OH-APAP	4-Acetamido-1,2-catechol
	3-OMe-APAP	2-O-Methyl-4-acetamido-1,2-catechol
	2-APBQ	2-Acetamido-*p*-benzoquinone
	4-AOBQ	4-Acetamido-*o*-benzoquinone
	MAPQI	N-Acetyl-3-methoxy-*p*-benzoquinone imine

good sensitivity levels. Herein, we report on the use of TSP LC–MS in a drug metabolism study to detect and characterize cysteine, N-acetylcysteine and glucuronide metabolites of 3'-hydroxyacetonilide (AMAP; see Table I for structures and abbreviations).

AMAP is a non-hepatotoxic regioisomer of acetaminophen (4'-hydroxyacetanilide; APAP)[12,13]. Despite the lack of hepatotoxicity of AMAP, when equal doses of radiolabeled AMAP and APAP (400 mg/kg) were administered to hamsters, the extent of covalent binding of radioactivity to hepatic proteins was found to be nearly the same for both compounds[3]. Furthermore, NADPH-dependent covalent binding of radioactivity from [^{14}C]AMAP to microsomes from phenobarbital-pretreated mice ocurred four times faster than covalent binding of radioactivity from [^{14}C]APAP. Although the binding could be blocked by the addition of glutathione (GSH), little GSH depletion was observed after the administration of AMAP to control hamsters and mice[12,13]. Thioether adducts have been searched for, but not detected, as urinary metabolites in either animal species[14,15].

AMAP is metabolized *in vitro* to diphenolic compounds, 2-acetamidohydroquinone (2-AcHQ) and 3-hydroxyacetaminophen (3-OH-APAP)[5]. *In vivo*, 3-OH-APAP is further methylated to give 3-methoxyacetaminophen (3-OMe-APAP)[4]. 2-AcHQ is oxidized further to 2-acetamido-*p*-benzoquinone (2-APBQ) *in vitro* by mouse liver microsomes[16,17].

In a previous study[18], we presented evidence for the formation of three different reactive intermediates as a result of AMAP oxidative metabolism. Both *ortho*- and *para*-quinones are formed by further oxidation of 3-OH-APAP and 2-AcHQ. A quinone imine, N-acetyl-3-methoxy-*p*-benzoquinone imine (MAPQI) is formed by further oxidation of 3-OMe-APAP. All three intermediates form glutathione conjugates that were detected *in vitro* in mouse microsomal incubations enriched with GSH and in the bile of mice treated with AMAP.

In view of the data described above, it became necessary to study the urinary disposition of AMAP and compare it with that of APAP. This required the synthesis and characterization of several of cysteine (CYSH) and N-acetylcysteine (NACYSH) conjugates of the reactive intermediates.

MATERIALS AND METHODS

AMAP was purchased from Aldrich (Milwaukee, WI, U.S.A.) and crystallized before use from ethanol–water (1:1). CYSH, NACYSH were obtained from Sigma (St. Louis, MO, U.S.A.). An authentic sample of 3-OMe-APAP glucuronide was a generous gift from Dr. M. W. Gemborys.

Instrumentation

High-performance liquid chromatography (HPLC) was performed on an LKB Model 2152-2SD dual-pump instrument equipped with an LKB Model 2152 LC controller and an LKB Model 2151 variable-wavelength detector used at 254 nm (LKB, Bromma, Sweden). Separations were carried out on a 5-μm Ultrasphere ODS column (25 cm × 10 mm I.D.) protected with a 5-μm Ultrasphere ODS precolumn (Rainin Instruments). Mobile phase consisted of solvent A: water–acetonitrile–glacial acetic acid (96:2:2, v/v/v); solvent B: acetonitrile. A linear gradient was employed from 0% B to 20% B in 40 min with a flow-rate of 2.0 ml/min.

LC–MS spectra were generated using an LKB Model 2150 pump with an LKB Model 2152 LC controller coupled to a Vestec thermospray LC–MS Model 201 dedicated LC–MS system (Vestec, Houston, TX, U.S.A.). The separation was carried on a 5-μm Ultrasphere ODS (15 cm × 4.6 mm I.D.) protected with a 5-μm Ultrasphere ODS precolumn. The mobile phase consisted of 0.1 *M* ammonium acetate–acetonitrile (90:10, v/v) pumped isocratically at a flow-rate of 1.0 ml/min. Operating temperatures for the TSP interface were T1 (vaporizer) = 130–140°C; T2 (tip) = 250–270°C; jet (vapor) = 300–320°C; source block = 320–350°C. The system was operated in the positive ion mode with the filament off.

The thermospray interface temperature was optimized to obtain the maximum intensity for the MH^+ ion of the synthetic standards. Although a lower source temperature produced less fragmentation, the overall sensitivity was reduced significantly. Full spectra of the synthetic standards were obtained by injecting 0.5–5 μg on column. The abundance of the MH^+ ion was observed to be higher at the top end of the range.

Animals and treatments

Male Swiss-Webster mice (16–25 g) were administered sodium phenobarbital (0.1% solution) as drinking water for 5 days. Animals were fasted 12 h prior to i.p. administration of AMAP (600 mg/kg) dissolved in warm saline. Urine was collected for 24 h in cups containing ascorbic acid and immersed in dry ice/acetone. Urine was first filtered through cotton wool then through a 0.45-μm Nylon-66 filter and frozen at −20°C until analyzed.

Preparations of standards

2-AcHQ[16], 3-OH-APAP[19], 3-OMe-APAP[19], 4-acetamido-*o*-benzoquinone (4-AOBQ)[20], 2-APBQ[17] and MAPQI[18] were synthesized as previously described. The following is the general procedure used for the preparation of the thioether conjugates. In a 5-ml Reacti-vial, CYSH or NACYSH (0.12 mmol) was dissolved in 2.5 ml phosphate buffer (pH 7.4) and sealed under nitrogen. The quinone or quinone imine (0.1 mmol) was dissolved in 1 ml acetonitrile (HPLC grade) and injected gradually through the septum of the vial under nitrogen. The reaction mixture was stirred for 30 min, acidified with formic acid to pH 2.0 and the acetonitrile removed using a gentle stream of nitrogen. The aqueous solutions were frozen at −20°C until HPLC purification was performed. Following purification and lyophyllization, the powders obtained were stored at −20°C. For NMR purposes, the powders were dissolved in ^2H_2O and lyophillized once more.

RESULTS

Synthesis of the mercapturic acid conjugates of 4-AOBQ

4-AOBQ when allowed to react with NACYSH gave several products as determined by HPLC–UV analysis and LC–MS. ^1H NMR analysis (Fig. 1) of the major product purified by HPLC revealed that this material was 3-(N-acetyl-cystein-S-yl)-5-acetamidocatechol. The CYSH β-methylene protons appeared as two sets of doublets of doublets at δ 3.16 and 3.42, while the α-proton appeared as a doublet of doublets at δ 4.37. The splitting pattern for the CYSH α and β protons is

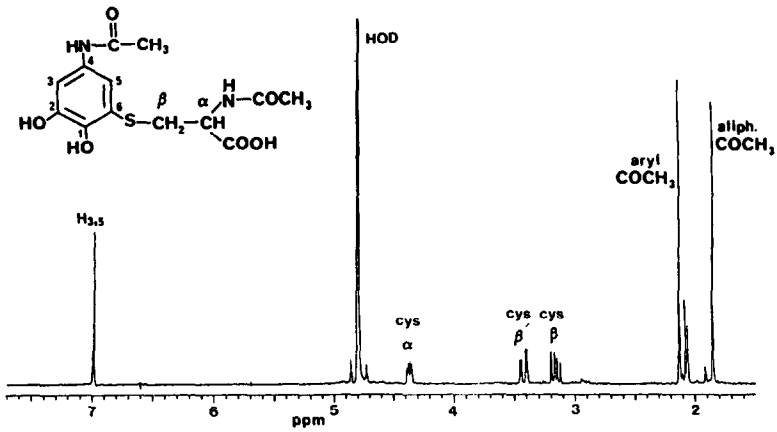


Fig. 1. ^{1}H NMR spectrum of the synthetic conjugate of 4-AOBQ.

characteristic of an ABX system. The methyl protons of the acetyl side chain of NACYSH appeared as a singlet at δ 1.85 and integrated for three protons. The methyl protons of the aryl acetamido group appeared as a singlet at δ 2.13 and integrated for three protons. The singlet at δ 6.98 integrated for two protons. These two aromatic protons were assigned to carbons 3 and 5 of the aromatic nucleus analogous to the glutathione conjugate of 4-AOBQ[18] which appear as two doublets with a coupling constant of 2.3 Hz.

The mercapturic acid conjugate was characterized further by TSP LC–MS (Fig. 2A). The spectrum showed a strong protonated molecular ion (MH^+) at *m/z* 329 and an ammonium adduct at *m/z* 346. In addition the spectrum showed several fragment ions at *m/z* 311, 287, 259, 200, 168, 147, 130. These ions are explained as follows (*m/z*): loss of H_2O (311), loss of ketene to give the CYSH conjugate (287), loss of H_2O from the CYSH conjugate (259), loss of N-acetyldehydroalanine to give the protonated catechol thiol (200), loss of N-acetyldehydrocysteine to give the protonated catechol compound (168), ammoniated N-acetyldehydroalanine (147), loss of H_2S from protonated NACYSH (130). Two other ions in the spectrum at *m/z* 217 and 182 are related to the base peak at *m/z* 200, the first being an ammonium ion adduct of the thiol compound and the other formed by elimination of water, respectively. This type of fragmentation to give the characteristic ions at *m/z* 200 and 168 have been observed previously in the spectra of the CYSH, NACYSH, and GSH conjugates of APAP (*m/z* 184, 152) under CI-MS[8] and TSP LC–MS[21] conditions. Under FAB-MS conditions, the CYSH and NACYSH conjugates of APAP essentially showed protonated molecular ions and sodiated molecular ions[9]. The fragment ion at *m/z* 287 is believed to be due to loss of ketene from the N-acetyl side chain and not from the aromatic acetamido group because the parent compound AMAP does not lose ketene to any significant extent.

A minor product of the reaction was the reduction product, 3-OH-APAP, as determined by co-chromatography with the synthetic standard and by LC–MS of the reaction mixture. Another minor product that eluted earlier on HPLC than the mercapturic acid conjugate was tentatively characterized as a *bis*-N-acetylcysteine conjugate of 4-AOBQ based on its LC–MS spectrum. The spectrum showed

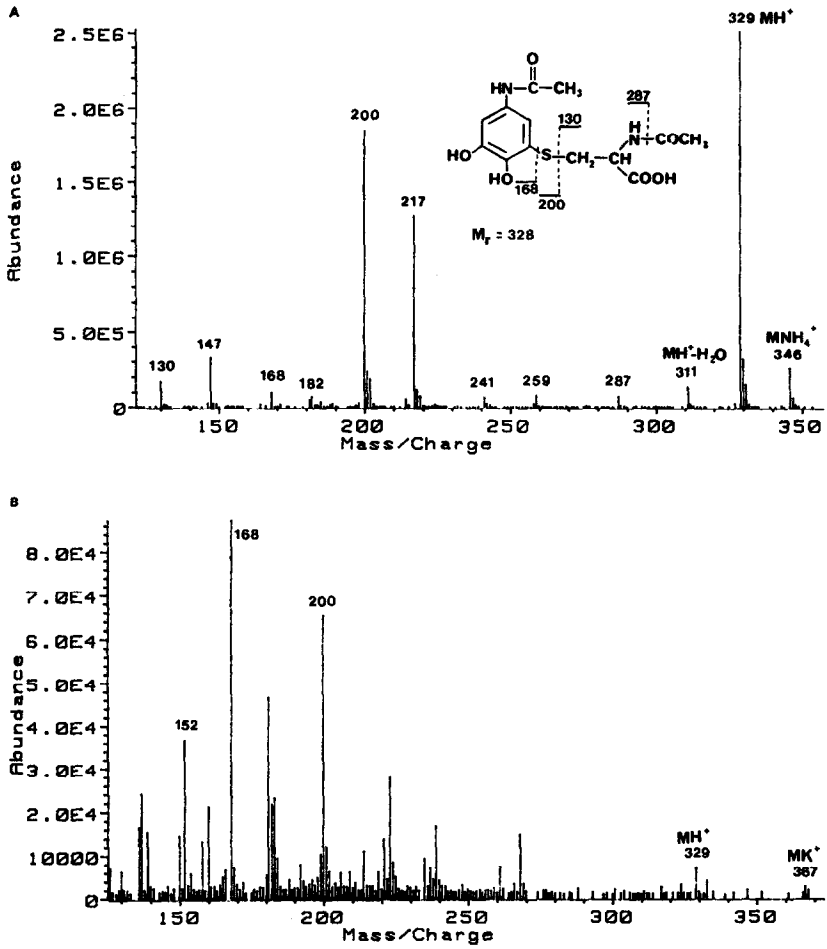


Fig. 2. (A) TSP LC–MS spectrum of the synthetic NACYSH conjugate of 4-AOBQ. (B) TSP LC–MS spectrum of the NACYSH conjugate of 4-AOBQ found in urine of mice given AMAP.

a relatively weak MH^+ at *m/z* 490 and ions at *m/z* 472 and 361 which would correspond to loss of H_2O and N-acetyldehydroalanine, respectively. Other characteristic ions were observed at *m/z* 329, 200, 182, 168, 130, the origin of which were explained above. Not enough material was on hand to rigorously characterize this compound further.

Synthesis of the cysteine conjugates of 4-AOBQ

When 4-AOBQ was allowed to react with CYSH, it gave one major adduct and a small amount of 3-OH-APAP. The reaction mixture was analyzed directly by LC–MS. The spectrum obtained for the CYSH adduct was very similar to that of the corresponding mercapturate except that the MH^+ was at *m/z* 287. Other characteristic ions were observed at *m/z* 217, 200, 168. No ions at *m/z* 146 or 130 were observed since these could only arise from the N-acetylcysteine type conjugates.

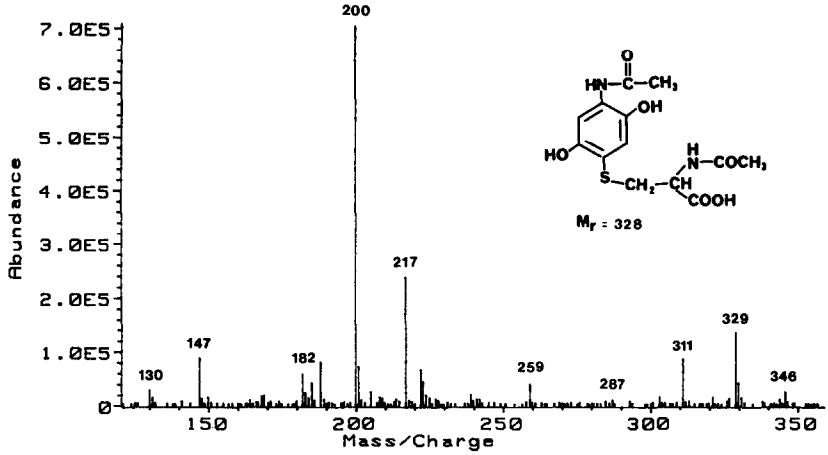


Fig. 3. TSP LC–MS spectrum of the synthetic NACYSH conjugate of 2-APBQ.

Synthesis of the mercapturic acid conjugates of 2-APBQ

When 2-APBQ was allowed to react with NACYSH, it gave several products that were characterized by LC–MS. Two major components of the reaction that were slightly resolved under the LC–MS conditions (see Experimental section) were characterized as *mono*-NACYSH conjugates of 2-APBQ. The spectrum in Fig. 3 is representative of these two compounds. Essentially the spectrum is very similar to that of the mercapturic acid conjugate of 4-AOBQ and showed the same ions discussed above. However, the 2-APBQ adduct eluted later in the chromatogram than that of the 4-AOBQ adduct. The other minor components that were observed in the analyzed mixture were the reduced quinone, 2-AcHQ, and what is tentatively characterized as a *bis*-N-acetylcysteine conjugate. This conjugate also eluted earlier than the *mono*-conjugates. The spectrum (Fig. 4) showed a MH^+ at m/z 490. Other characteristic ions

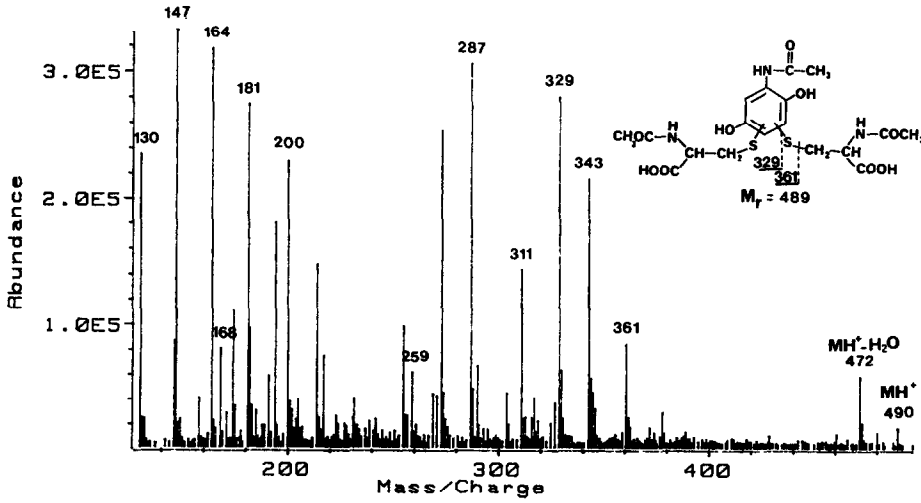


Fig. 4. TSP LC–MS spectrum of the synthetic *bis*-NACYSH conjugate of 2-APBQ.

were observed at *m/z* 472, 361, 343, 329, 311, 287, 217, 200, 168, 147, 130. Except for the ion at *m/z* 343 (loss of H_2O from ion at *m/z* 361), the origins for these ions were explained above for the 4-AOBQ *bis*-adduct. The spectrum also showed ions that would result from thermally formed NACYSH at *m/z* 181, 164, 146 which correspond to an ammonium ion adduct, protonated NACYSH, and loss of the elements of water, respectively. An authentic sample of NACYSH eluted earlier in the chromatogram, yet gave the same ions.

Synthesis of the cysteine conjugates of 2-APBQ

CYSH reacted with 2-APBQ to give the reduction product and two cysteine conjugates that were resolved under LC–MS conditions. Both conjugates gave the same ions observed for the CYSH conjugate of the regioisomer 4-AOBQ and had longer retention times.

Synthesis of the mercapturic acid conjugates of N-acetyl-3-methoxy-p-benzoquinone imine (MAPQI)

MAPQI underwent reaction with NACYSH to give two major products, 3-OMe-APAP and another product that was found to be a mercapturate following ^1H NMR and LC–MS analysis. The NMR spectrum (Fig. 5) showed three singlets at δ 1.82, 2.15, and 3.87, each integrated for three protons. These correspond to the acetyl protons of the side chain, the aryl acetamido group protons, and the 3-methoxy group protons, respectively. The CYSH β-methylene protons appeared as two sets of doublets of doublets at δ 3.12 and 3.45. The CYSH α-proton appeared as a doublet of doublets at δ 4.28. The aromatic protons appeared as two singlets at δ 7.02 and 7.11. The resonance of these protons as singlets suggests a *para* relationship. However, the broadness of the singlets suggests a small coupling which has not been observed for para protons for these type of compounds. In addition, both GSH[18] and CYSH (see below) add to MAPQI *meta* to the acetamido group, thereby giving rise to *meta* protons on carbons 2 and 6 of the conjugates. Therefore, the protons in this

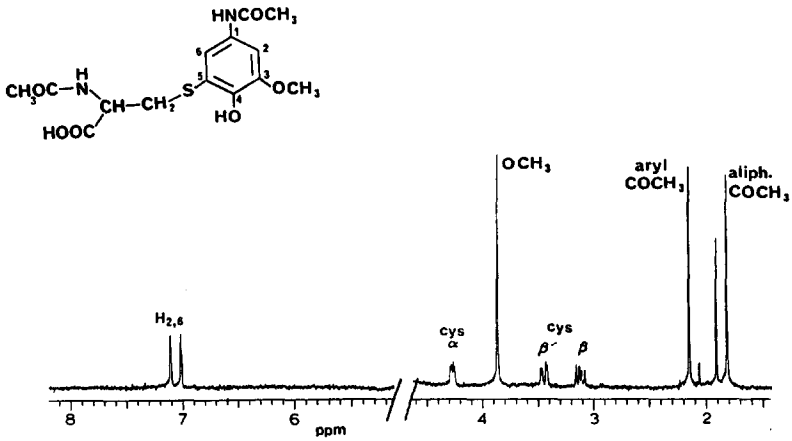


Fig. 5. ^1H NMR spectrum of the synthetic NACYSH conjugate of MAPQI.

mercapturic acid conjugate were assigned to the same carbons, and it is proposed that the compound is 3-(N-acetylcystein-S-yl)-5-methoxyacetaminophen. This compound was characterized further by LC–MS (Fig. 6A). The spectrum shows the MH^+ ion m/z 343 and other characteristic ions at m/z 301, 214, 228, 182. These correspond to losses of ketene, N-acetyldehydroalanine, N-acetyldehydroglycine, and N-acetyldehydrocysteine, respectively.

Synthesis of the cysteine conjugates of MAPQI

CYSH, when allowed to react with MAPQI also gave 3-OMe-APAP and a CYSH conjugate that was purified with HPLC. ^1H NMR (Fig. 7) indicated that this conjugate was 3-(cystein-S-yl)-5-methoxyacetaminophen. The assignement of the CYSH moiety to carbon 3 on the aromatic ring is based on the appearance of two

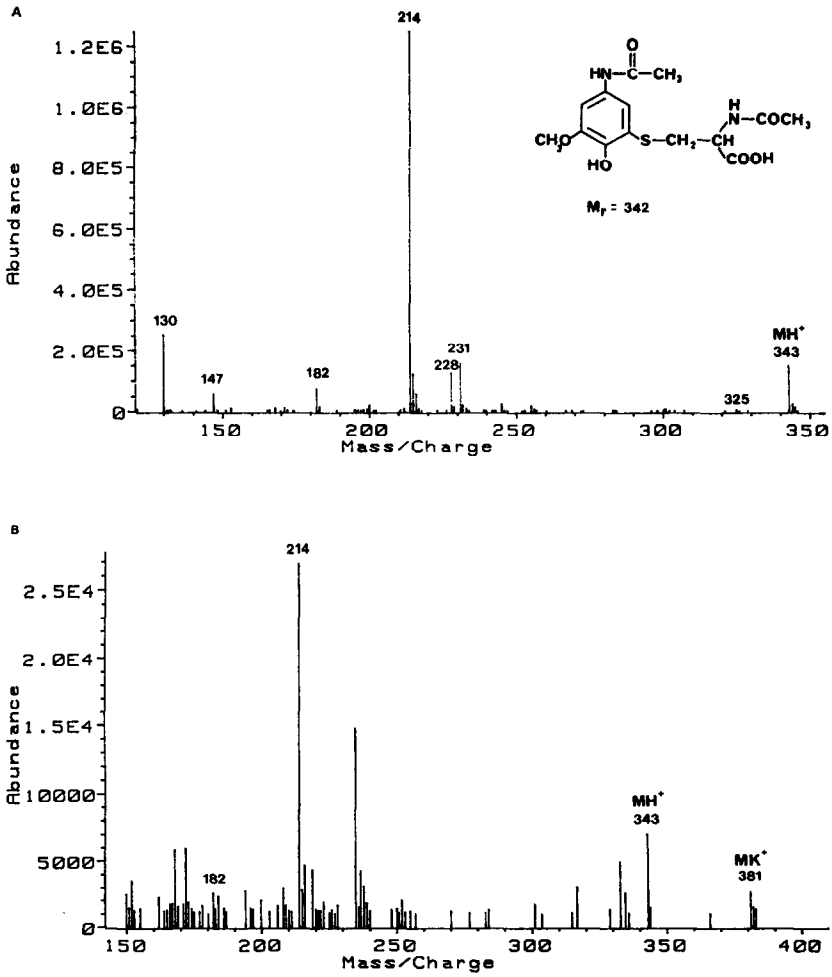


Fig. 6. (A) TSP LC–MS spectrum of the synthetic NACYSH conjugate of MAPQI. (B) TSP LC–MS spectrum of the synthetic NACYSH conjugate of MAPQI found in urine of mice given AMAP.

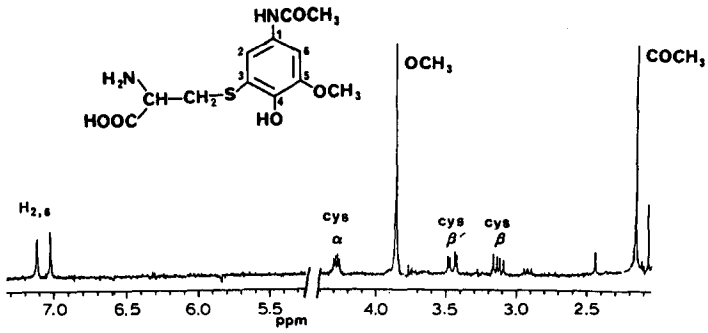


Fig. 7. ^{1}H NMR spectrum of the synthetic CYSH conjugate of MAPQI.

doublets at δ 7.02 and 7.12, each with a coupling constant of 2.3 Hz, indicating a *meta* relationship. Since the position of the 3-methoxy group is predefined, we assigned those protons to carbons 2 and 6, and hence the CYSH moiety must be attached to carbon 3. Further characterization of this compound was performed by LC–MS analysis. The spectrum of this compound exhibited an MH^+ ion at *m/z* 301 and the characteristic ions seen with the N-acetyl derivative, namely ions at *m/z* 214 and 228.

Studies in urine of mice treated with AMAP

The synthetic standards of the thioether conjugates of AMAP metabolites were used to develop an HPLC gradient elution program (see Experimental section) that gave baseline separation of these compounds as well as for AMAP, 3-OMe-APAP, 3-OH-APAP, 2-AcHQ. Table II provides the retention times for these compounds. When urine obtained from mice treated with AMAP (600 mg/kg) was analyzed under these conditions, UV peaks were observed at the retention times for the thioether adducts. Fig. 8 shows the chromatogram observed. The proposed identity of the peaks corresponding to the thioether conjugates was supported by co-chromatography with the standards.

TABLE II

HPLC RETENTION TIME DATA OF AMAP AND ITS KNOWN METABOLITES

Compound name	*Peak No.*	*Retention time (min)*
AMAP glucuronide	1	17.2
3-OMe-APAP glucuronide	2	17.5
AMAP	3	25.0
3-OMe-APAP	4	24.6
3-OH-APAP	5	15.8
2-AcHQ	6	16.3
CYCSH of AOBQ	7	19.0
NACYSH of AOBQ	8	23.5
CYSH of APBQ	9, 10	20.8, 21.8
CYSH of MAPQI	11	26.8
NACYSH of MAPQI	12	13.8

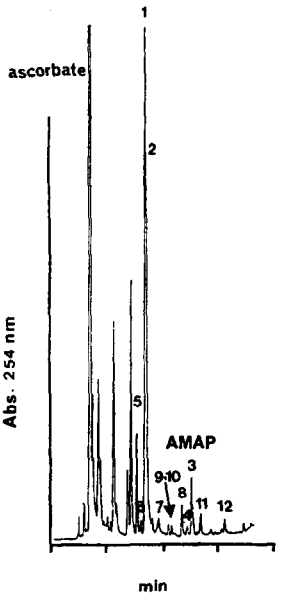


Fig. 8. HPLC analysis of urine obtained from mice treated with 600 mg/kg AMAP.

LC–MS analysis of urine

To further support conclusions described above, untreated urine was analyzed by TSP LC–MS under the same conditions used for analysis of the synthetic standards. Fig. 9 shows the total ion chromatogram (TIC) obtained. A search for the thioether adducts derived from AMAP was done by reconstructing ion chromatograms for the expected MH^+ ions and the base peak ions established for the standards. We were able to detect the NACYSH conjugate of AOBQ (Fig. 2B) and the NACYSH conjugates of MAPQI (Fig. 6B) as well as the CYSH conjugate of the latter. The major peak in the

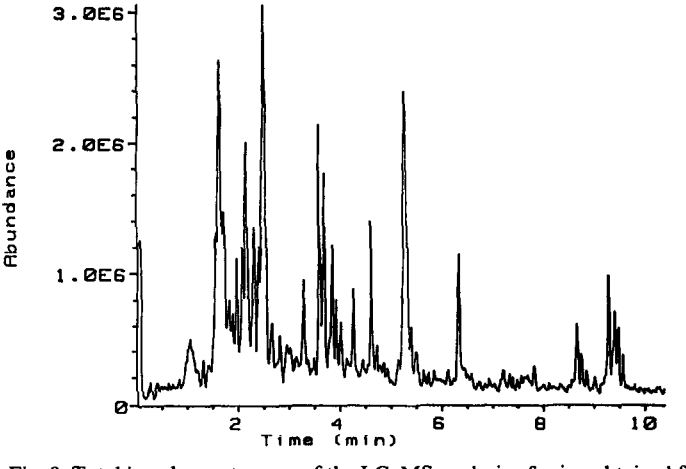


Fig. 9. Total ion chromatogram of the LC–MS analysis of urine obtained from mice treated with AMAP.

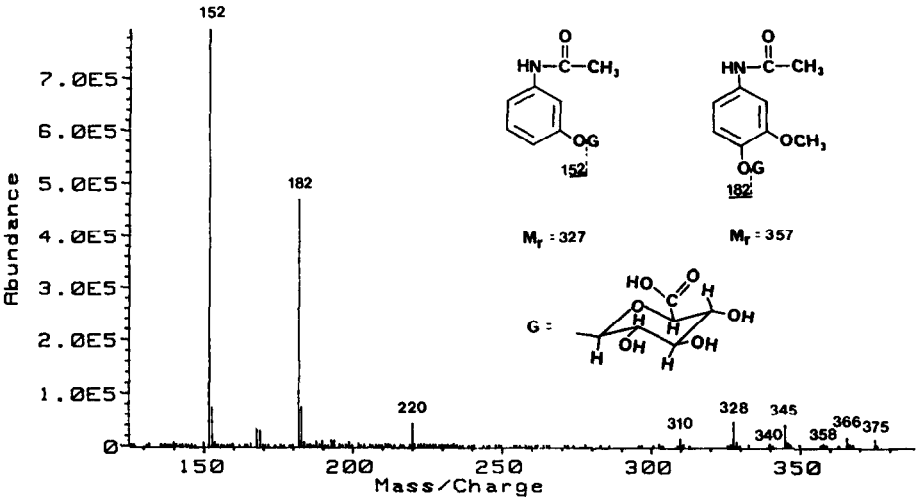


Fig. 10. TSP LC–MS spectrum of a mixture of AMAP glucuronide and 3-OMe-APAP glucuronide excreted in urine of mice given AMAP.

TIC (2.5 min) was found to correspond to a mixture of AMAP glucuronide and 3-OMe-APAP glucuronide that are co-eluting. The TSP mass spectrum of these two conjugates is shown in Fig. 10. The ions from the AMAP glucuronide were more dominant in the spectrum and they appeared at *m/z* 366 [MK^+], 350 [MNa^+], 345 [MNH_4^+], 328 [MH^+], 310 [$MH^+ - H_2O$], and 152 [MH^+ – dehydroglucuronic acid]. The ions from the 3-OMe-APAP glucuronide appeared at *m/z* 375 [MNH_4^+], 358 [MH^+], 340 [$MH^+ - H_2O$], and 182 [MH^+ – dehydroglucuronic acid]. When the authentic sample of the 3-OMe-APAP glucuronide isolated from bile of rabbits given acetaminophen was injected under the same conditions, it had the same retention time and gave the same ions observed for that present in AMAP urine. The identity of these two conjugates was also supported by their disappearance from the UV chromatograms following β-glucuronidase hydrolysis (data not shown). Glucuronide conjugates of the diphenolic metabolites of AMAP were also detected as a major component in the urine. TSP LC–MS spectrum of these components showed ions at *m/z* 382 [MK^+], 344 [MH^+], 326 [$MH^+ - H_2O$], and 168 [MH^+ – dehydroglucuronic acid]. The loss of the elements of water and that of neutral dehydroglucuronic acid (176 a.m.u.) is characteristic for this type of metabolites under TSP conditions[2,21,22].

CONCLUSION

Collectively the results presented above support previous work that indicated that AMAP is metabolized *in vivo* to three reactive intermediates, 2-APBQ, 4-AOBQ, and MAPQI. These intermediates are similar to NAPQI, the toxic metabolite from APAP, in being trapped as thioether adducts in the presence of GSH and metabolic breakdown products thereof are excreted in urine as CYSH and NACYSH adducts. Fig. 11 depicts the metabolic profile of AMAP in the mouse.

In order to investigate the importance of the reactive metabolite pathways in the

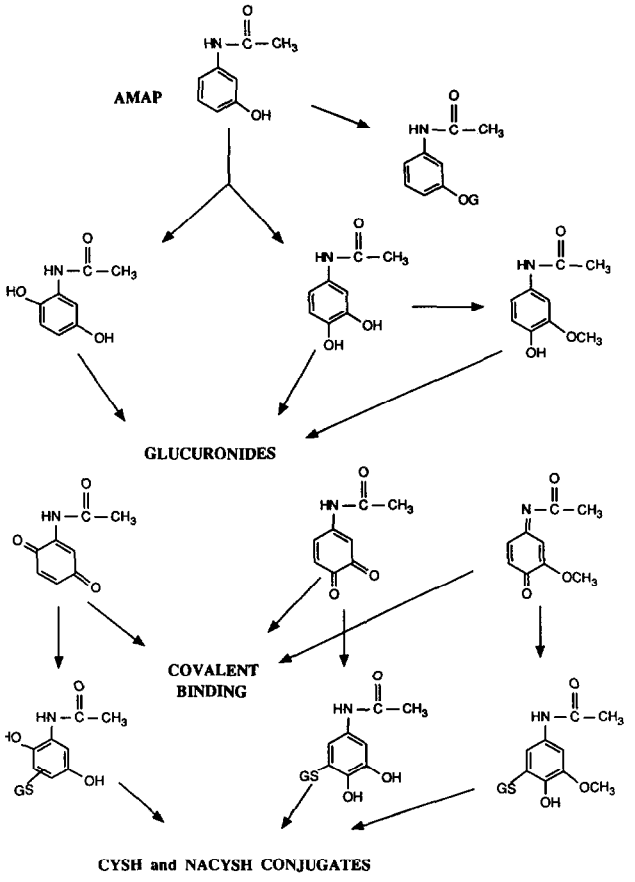


Fig. 11. Metabolic profile for the metabolism of AMAP in the mouse.

overall dispositon of AMAP, we synthesized appropiate standards, developed HPLC methods for separating the standards and utilized both NMR and MS techniques to characterize these compounds. The use of TSP LC–MS was critical in the characterization of the synthetic thioether standards because of their water solubility and their relative instability due to autooxidation especially of the hydroquinone type adducts that in some cases hampered isolation of the materials for NMR and direct mass spectral characterization by other soft ionization techniques such as FAB-MS. In addition, TSP spectra provided several characteristic fragmentation ions that aided in the unequivocal identification of the standards as well as the metabolites in urine. Work is in progress to determine the quantitative importance of the metabolic activation of AMAP in the overall disposition of AMAP.

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REFERENCES

1 C. R. Blakely, J. J. Carmody and M. L. Vestal, *J. Am. Chem. Soc.*, 102 (1980) 5931.
2 D. J. Liberato, C. C. Fenselau, M. L. Vestal and A. L. Yergey, *Anal. Chem.*, 55 (1983) 1741.
3 R. D. Voyksner and E. D. Bush, *Biomed. Environ. Mass. Spectrom.*, 14 (1987) 213.
4 T. A. Bellar and W. L. Budde, *Anal. Chem.*, 60 (1988) 2076.
5 C. E. M. Heeremans, R. A. M. van der Hoeven, W. M. A. Niessen, U. R. Tjaden, G. F. La Vos and J. van der Greef, *Biomed. Environ. Mass. Spectrom.*, 17 (1988) 377.
6 M. S. Lan, J. Oxford and L. E. Martin, *J. Chromatogr.*, 394 (1987) 223.
7 J. N. H. Meerman, F. E. Beland, B. Ketterer, S. K. S. Srai, A. P. Baruins and G. J. Mulder, *Chem. Biol. Interactions,* 39 (1982) 149.
8 S. D. Nelson, Y. Vaishnav, H. Kambara and T. A. Baillie, *Biomed. Mass. Spectrom.*, 8 (1981) 244.
9 B. L. Ackerman, J. T. Watson, J. F. Newton, Jr., J. B. Hook and W. E. Braselton, Jr., *Biomed. Environ. Mass. Spectrom.*, 11 (1984) 502.
10 C. E. Parker, J. S. M. de Wit, R. W. Smith, M. B. Gopinathan, O. Hernandez, K. B. Tomer, C. H. Vestal, J. M. Sanders and J. R. Bend, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 623.
11 A. Conchillo, J. Casas, A. Messeguer and J. Abián, *Biomed. Environ. Mass Spectrom.*, 16 (1988) 339.
12 S. A. Roberts and D. J. Jollow, *Fed. Proc.*, 38 (1979) 426.
13 E. B. Nelson, *Res. Commun. Chem. Pathol. Pharmacol.*, 28 (1980) 447.
14 S. A. Roberts and D. J. Jollow, *Pharmacologist*, 2 (1978) 259.
15 M. Hamilton and P.T. Kissinger, *Drug. Metab. Dispos.*, 14 (1986) 5.
16 A. J. Streeter, S. M. Bjorge, D. B. Axworthy, S. D. Nelson and T. A. Baillie, *Drug Metab. Dispos.*, 12 (1984) 565.
17 A. J. Streeter and T. A. Baillie, *Biochem. Pharmacol.*, 34 (1985) 2871.
18 M. S. Rashed and S. D. Nelson, *Chem. Res. Toxicol.*, 2 (1989) 41.
19 A. J. Forte, J. M. Wilson, J. T. Slattery and S. D. Nelson, *Drug Metab. Dispos.*, 12 (1984) 484.
20 M. F. Ansell, A. F. Bignold, A. F. Gosden, V. J. Leslie and R. A. Murray, *J. Chem. Soc. C,* (1971) 1414.
21 L. D. Betowski, W. A. Korfmacher, J. O. Lay, Jr., D. W. Potter and J. A. Hinson, *Biomed. Environ. Mass Spectrom.*, 14 (1987) 705.
22 C. Fenselau and L. Yellet, in G. G. Paulson, J. Caldwell, D. H. Huston and J. J. Menn (Editors), *Xenobiotic Conjugation Chemistry, ACS Symp. Ser.*, 299 (1986) 159.