peak in hexane (472–475 nm) and was entirely hypophasic when partitioned between hexane and 95% methanol. The carotenoid from *N. ornatus* was, therefore, identified as esterified astaxanthin which after saponification yielded astacene.

Although all the different species of Acanthocephala so far investigated do not possess the same carotenoids, each species appears to contain a single major carotenoid and not a mixture of pigments. There are, with the possible exception of the mammalian corpus luteum[12] no authenticated cases of de novo carotenoid synthesis in animals, so the parasites must obtain their carotenoids from their hosts. However, the carotenoids found in adult acanthocephalans do not necessarily appear to reflect the spectrum of carotenoids available to the parasite from their hosts diet.

There seems to be no correlation between the type of intermediate host and the carotenoid found in the adult parasite, although the majority of coloured Acanthocephala have Crustacea as their intermediate host. In a secondary host, such as *Gammarus pulex*, a wide range of carotenoids are present[1] although astaxanthin forms the major carotenoid available in the haemolymph[13]. Yet the adults of the 2 species of Acanthocephala which utilize this intermediate host, namely *P. laevis* and *P. minutus* contain different carotenoids. Possibly there is a correlation between the diet of the final host and the carotenoid present in the adult acanthocephalan.

The uptake of carotenoids by adult acanthocephalans would appear to be selective, since they only concentrate one of the available carotenoids. Selective uptake of carotenoids has been reported in certain other invertebrate groups[14]; some polychaetes contain only β-carotene and do not appear to absorb hydroxycarotenoids[15], whilst certain molluscs show a preferential uptake of xanthophylls[16]. The uptake of carotenoids by adult Acanthocephala may be associated with the uptake of other compounds, such as lipids and/or proteins in the form of lipocarotenoid complexes or carotenoproteins. In *P. minutus* cystacanths the esterified astaxanthin is associated with wax esters which constitute nearly 90% of the total cystacanth lipids[17].

The role of carotenoids in adult Acanthocephala is obscure, carotenoids may have an anti-oxidant function[14] or they may be involved in oxidative metabolism[18]. Alternatively the vitamin A function of carotenoids could be important in Acanthocephala, or carotenoids may be involved in reproduction[14]. However, carotenoids do not appear to be essential to the adult acanthocephalan parasite[1] and the concentration of carotenoids can vary widely from individual to individual and is completely absent in many species.

Whether the distribution of α- and β-carotene and their derivatives in the different classes of Acanthocephala has any phylogenetic significance is not known. β-Carotene and its derivative astaxanthin occur only in Palaeacanthocephala, whilst lutein (an α-carotene derivative) is found in the Archiacanthocephala and Eoacanthocephala. This may represent a biochemical difference between the 3 classes of Acanthocephala or merely reflect the ecological differences of their hosts.

Zusammenfassung. Die Carotinoide in 6 Spezies erwachsener Acanthocephalen wurden identifiziert. Lutein wurde als einziges Carotinoid in *P. galaxis* und *N. pseudemydis* gefunden und als wichtigstes Carotinoid in *M. hirudinaceus* festgestellt. *F. anatis* und *P. laevis* enthielten nur β-Carotin, während das veresterte Astexanthin als einziges Pigment in *N. ornatus* auftrat.

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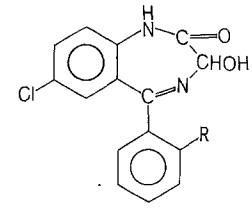
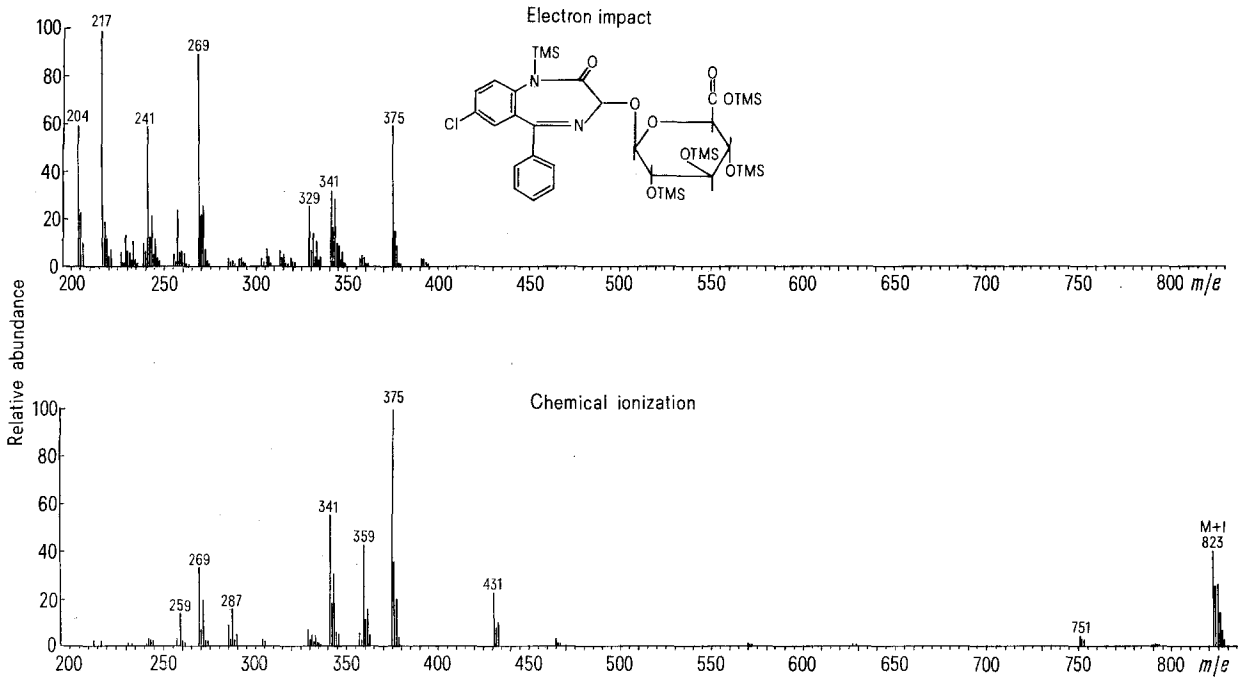
Identification of Oxazepam and Lorazepam Glucuronides by Chemical Ionization Mass Spectrometry

Oxazepam (7-chloro-3-hydroxy-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one) and lorazepam (7-chloro-5-(*o*-chlorophenyl)-3-hydroxy-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one) are benzodiazepines in clinical use by virtue of their antianxiety, tranquilizing and sedative properties. Their respective glucuronide conjugates are the major urinary metabolites in man and in several other species[1–3]; they also circulate together with the free drugs in human blood[4,5]. Furthermore, oxazepam glucuronide is a metabolite of diazepam[6,7] prazepam[8] temazepam[9] and medazepam[10,11]. In view of their presence in blood and their importance as final detoxification products it is highly desirable to obtain conclusive evidence for the structure of these conjugates which so far have been characterized only by enzymic cleavage. This communication describes the isolation, chemical analysis and identification by chemical ionization (CI) mass spectrometry[12] of the glucuronides of oxazepam and lorazepam.

Isolation of the metabolites was accomplished by first processing on XAD-2 resin urines from miniature swine orally dosed with 50 mg/kg oxazepam or lorazepam. Elution from the resin was accomplished with 80% aqueous acetone. After evaporation of acetone it was further purified by adsorption on charcoal and subsequent



Oxazepam R=H
Lorazepam R=Cl



Mass spectra of silylated oxazepam glucuronide.

elution with 80% aqueous acetone. The aqueous solution remaining after the evaporation of acetone was extracted with ethyl acetate to remove all freely extractable substances. DEAE-Cellulose (Whatman-DE 23) chromatography of the remaining aqueous phase provided a final purification of the isolated conjugates of oxazepam and lorazepam. Experimental conditions included adjustment of the aqueous phase to pH 5 with 1 *N* NH_4OH, application to the prewashed anion-exchanger, elution with a concave gradient (0.0–0.29 *M* ammonium acetate buffer, pH 5), and finally lyophilization of the fractions containing the conjugates (as determined by UV-absorbance at 315 nm and thin-layer chromatography on cellulose).

The glucuronic acid content of the final preparations was determined by a modified carbazole method[13], and the benzodiazepinone moiety by gas liquid chromatography[4,5]. The molar ratio of parent drug to glucuronic acid was 1 : 1 for both isolates. Furthermore, the quantitative determination of the benzodiazepinone moieties indicates that the preparations were at least 85% pure. Since they were chromatographically (ion exchange as well as thin-layer chromatography) pure the only other substance present in significant amounts appeared to be water. Infrared spectrophotometry showed absorption mainly due to the glucuronic acid moiety. Significantly, absorptions in the carbonyl region (5.9 and 6.2 μ) were indicative of free carboxylic acid (as opposed to ester absorptions) thus indicating binding through an acetal link and not through ester formation.

Mass spectrometric analysis, however, was to provide the major means for the verification of structure. The instrument used was an AEI MS902 high resolution mass spectrometer (AEI Scientific Apparatus, Inc., Manchester, England) equipped with a Chemspect CIS-2 CI-EI source (Scientific Research Instruments Corp., Baltimore, Md.). The high resolution results were processed by a PDP-8 computer (Digital Equipments Corp., Maynard, Mass.). EI-spectra were obtained at 70eV. CI-spectra were obtained at 500 eV using isobutane as reagent gas (0.5 torr.). Probe temperatures were approximately 300 °C for both EI and CI, and the source temparature was 250 °C. The metabolites decomposed thermally inside the instrument during analysis. For this reason, trimethylsilyl (TMS) derivatives of the metabolites were prepared with Sylon BTZ (Supelco Inc., Bellefonte, Pa.) prior to the mass spectrometric examination.

The electron impact (EI) mass spectrum of silylated oxazepam glucuronide showed no ions above *m/e* 400 (see Figure). Among the major fragments shown, *m/e* 375 [$(C_{15}H_{31}O_5Si_3$, $C_6H_4O_2(OTMS)_3$], 217 [$(C_9H_{21}O_2Si_2$, $C_3H_3(OTMS)_3$] and 204 [$(C_8H_{20}O_2Si_2$, $C_2H_2(OTMS)_2$] contain no N or Cl and, therefore are related to the glucuronide portion of the molecule. The *m/e* 204 and 217 are characteristic fragment ions of silylated sugars[14]. The *m/e* 341 [$(C_{18}H_{18}N_2OClSi$, $C_{15}H_9N_2OCl(TMS)$], 269

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($C_{15}H_{10}N_2OCl$) and 241 ($C_{14}H_{10}N_2Cl$) fragments are related to the benzodiazepinone moiety. The peak at *m/e* 341 consists of the oxazepam portion with one TMS group but without the acetal oxygen atom at C-3. The TMS group is most likely located at the N-1 position, which indicates that N-1 is not linked to the glucuronic acid portion. Thus, this peak at *m/e* 341 is an important fragment ion which proves that the link with the glucuronic acid is formed through the C-3 acetal oxygen rather than the N-1 nitrogen. The *O*-glucuronide structure is in agreement with the observation that oxazepam glucuronide is readily cleaved by β-glucuronidase[4].

The above fragments are characteristic of the two moieties of the molecule and on EI no ions indicative of the presence of intact conjugate were observed. The CI-spectra of the metabolite, on the contrary, exhibited an intense MH^+ ion at *m/e* 823 as shown in the Figure. This is the mass expected from the oxazepam glucuronide which has been penta-silylated. This finding verifies the oxazepam-glucuronic acid conjugation. The peak at *m/e* 751 is the MH^+ ion of the tetra-silylated glucuronide due to incomplete silylation; different silylating conditions change the relative intensity of this peak. It is interesting to note that the fragmentation of the silylated glucuronic acid portion is much simpler in the CI-spectrum. The peak at *m/e* 375 is the only major ion related to the silylated glucuronic acid portion, i.e., the characteristic EI fragment ions at *m/e* 217 and 204 are conspicuously absent in the CI-spectrum. All other major peaks, including 431, 359, 341, 287 and 269, show the chlorine isotopic peak, therefore are related to the oxazepam moiety. No metastable ions were found to substantiate any fragmentation pathway. Since no important metabolite-related ions were observed below *m/e* 200 from both EI- and CI-spectra, they not shown in the Figure.

Similarly, observation of the MH^+ ion of a penta-silylated product at *m/e* 857 verified the glucuronide conjugation of lorazepam. Partial silylation of lorazepam glucuronide also yielded peaks at *m/e* 713 and 785 due to its tri-silylated and tetra-silylated products, respectively. Again, *m/e* 375 is the only ion related to the silylated glucuronic acid portion.

The striking difference between the EI and CI results clearly demonstrates the advantage of CI mass spectrometry. CI is highly sensitive, yields intense MH^+ ions and is also relatively unaffected by sample purify. This technique undoubtedly will be employed with increasing frequency in various metabolic studies especially those involving metabolites previously undetectable by EI-mass spectrometry.

Zusammenfassung. Die Glucuronide des Oxazepams und Lorazepams wurden aus dem Harn von Zwergschweinen, denen die entsprechenden Benzodiazepine verabreicht wurden, isoliert. Die Metaboliten wurden durch Analyse der Spaltprodukte gekennzeichnet. Massenspektrometrie der Trimethylsilylderavite war für die Struktur der *O*-Glucuronide beweisend.

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Occurrence of Heparin or its Related Acidic Glucosaminoglycan in Human Aortic Tissue

Analytical studies of human aortic connective tissue established that the aortic acidic glycosaminoglycans (AGAG) consist of hyaluronic acid, chondroitin sulfate isomers and heparan sulfates[1,2]. However, there is no agreement about heparin in human aortic tissue, because there is only a trace of heparin, if any, in the tissue[1-4]. Since these arterial AGAG as well as heparin function physiologically as anti-coagulants[5], anti-lipemics[6] and anti-thrombogenics[7], it should be important to study the presence of heparin in aortic tissue. An appreciable number of mast cells as a source of heparin has been reported in the arterial connective tissues. In 1937, Jorpes[8] first described the presence of mast cells in pig arterial tissue from which he extracted heparin. In human arterial tissue, several investigators reported the existence of mast cells in relation to the pathogenesis of atherosclerosis[9-11]. The present paper reports the occurrence of heparin or its related glucosaminoglycan in human aortic tissue.

Materials and methods. Freshly obtained, pooled, human aortic samples from subjects ranging in age from 40 to 77 years were subjected to the preparation of aortic AGAG by the method previously described[12,13]. Human aortic intima and media layers were carefully separated from the adventitia and homogenized in a grinder. After the lipids were repeatedly extracted with methanol-chloroform (1:2, v/v) from the samples, proteolytic

Table I. Fraction pattern of acidic glycosaminoglycans in human aorta on a Dowex 1-X2 column by stepwise elution with increased NaCl concentration

Molarity of NaCl	0.5	1.25	1.75	3.0	Total
Acidic glycosaminoglycans					
as glucuronic acid (mg)	0.73	6.60	15.67	2.41	25.41
Percentage	2.9	26.0	61.6	9.5	100.0

The yield of applied acidic glycosaminoglycans was 29.6 mg as glucuronic acid.

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