C-Glucuronidation of the Acetylenic Moiety of Ethchlorvynol in the Rabbit

Abstract. *An acetylenic* C-*glucuronide of the sedative-hypnotic drug ethchlorvynol was isolated from rabbit urine as the major metabolite. The* C-*glucuronide represents a novel metabolic pathway for acetylenes and is a rare example of the formation of a carbon-glucuronide bond in mammalian systems.*

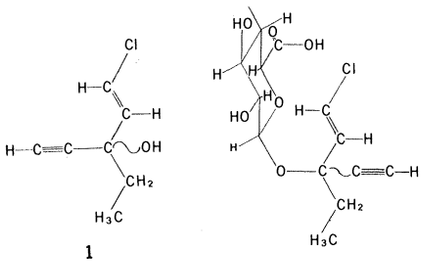
Glucuronidation is the major metabolic pathway for the elimination of many drugs and endogenous compounds. It is also the most common conjugation reaction in mammals (*1*). Indeed, a large variety of aromatic, aliphatic, and heterocyclic compounds containing hydroxyl, carboxyl, sulfhydryl, and amino or imino groups form glucuronides. In this report, we identify an acetylenic *C*-glucuronide of ethchlorvynol (structure **1**), a sedative-hypnotic drug. It is an example of an acetylenic conjugate. *C*-Glucuronides, conjugates of glucuronic acid attached by a carbon-carbon bond, are known for only two other types of compounds. The formation of *C*-glucuronides in man has been shown for the pyrazolidine drugs, phenylbutazone and sulfinpyrazone (*2*). In addition, an aromatic *C*-glucuronide of Δ^6-tetrahydrocannabinol has recently been observed in the mouse (*3*).

The metabolism of ethchlorvynol has been studied (*4, 5*) in man, rabbits, and rats. All of these species have been reported to metabolize **1** extensively (more than 95 percent). However, a glucuronide, sensitive to β-glucuronidase and accounting for 1 to 2 percent of a dose, has been the only metabolite identified. To further elucidate the metabolic fate of **1**, we administered ^{14}C-labeled **1** (custom synthesis, ICN, acetylenic-^{14}C, 1.05 Ci/mole) to rabbits and collected urine for analysis.

Essentially all the radioactive dose was found in the urine within 2 days after the administration into a marginal ear vein of a bolus (5 mg/kg) in isotonic saline. Less than 0.05 percent of the dose was excreted unchanged. Paper electrophoresis, ion-exchange chromatography, and extraction techniques indicated that the radioactivity in whole urine was a mixture of polar weak acid components. Our findings, which are consistent with those of Nitta *et al.* (*5*), were that only 1 to 2 percent of the urinary metabolites were hydrolyzed by sulfatase and β-glucuronidase (Glusulase, Endo Laboratories) to give a nonpolar extractable product.

The major metabolite in the urine (accounting for 30 percent of an intravenous dose of 60 mg/kg, 50 mCi/mole, infused during 5 hours) was isolated by high-performance liquid chromatography (*6*) with use of a C-18 reverse-phase column. Since the metabolites demonstrated no ultraviolet absorption at the wavelengths monitored (254, 280, and 365 nm), the effluent was sampled for radioactivity by liquid scintillation counting. Urine samples were concentrated by lyophilization, reconstituted in a small volume of appropriate mobile phase, and centrifuged to remove particulates. Three mobile phases were employed to effect separation and isolation: (i) to separate the major metabolite from other metabolites, 1 percent acetic acid, 30 percent methanol, and 2 percent acetonitrile; (ii) to separate the major metabolite from urinary components, 40 percent methanol in 10 m*M* acetate buffer, *p*H 6.0; and (iii) to further purify the major metabolite and remove salts, 5 percent methanol and 5 percent acetonitrile in water.

Mass spectra (*7*) were obtained after treating the isolated metabolite with diazomethane followed by *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (*8*) overnight at room temperature. With direct insertion, the electron-impact mass spectrum showed a fragmentation pattern typical of the carbohydrate portion of a glucuronide (*9*) at mass-to-charge ratios (*m/e*) of 407, 317, 275, 217, and 204. However, no molecular ion was seen, and the only chlorine-containing fragment was at *m/e* 127/129. A chemical ionization mass spectrum showed nothing at the MH^+ expected (*m/e* 551/553) for the methyl ester of a tris-trimethylsilyl derivative of ethchlorvynol *O*-glucuronide, structure **2**, but instead showed ions at *m/e* 623/625, 407, 317 (base peak), 275, and 127/129.



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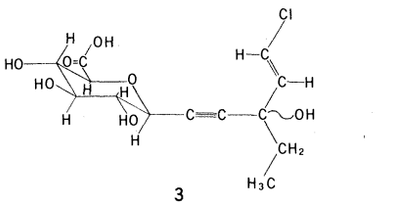

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The observation of an MH^+ ion 72 mass units higher than that expected for the *O*-glucuronide, structure **2**, suggested the incorporation of an additional trimethylsilyl (TMS) group during the derivatization. This was confirmed by derivatizing a fresh sample with diazomethane followed by *N*,*O*,-bis(trideuteromethylsilyl)trifluoroacetamide-d_{18} (*10*). The chemical ionization spectrum of this derivative showed that the MH^+ had moved 36 mass units to *m/e* 659/661, thus confirming the incorporation of four TMS groups. Some preparations also showed an ion at *m/e* 681/683, which corresponded to a derivative with five TMS groups minus a methylene, presumably a TMS ester formed because of incomplete methylation of the acid during the diazomethane step.

The mass spectral data were consistent with structure **3** (*11*), but could also be explained by structure **2** if the terminal hydrogen of the acetylene group were replaced by a TMS group. However, when ethchlorvynol was silylated under exactly the same conditions as those used for the metabolite, the infrared, proton magnetic resonance, and mass spectra of the product showed replacement of the alcoholic, but not of the acetylenic, hydrogen.

Conjugation with glucuronic acid at the acetylene position was further confirmed by infrared spectroscopy (*12*), with carbon tetrachloride solutions of **1**, the acetate of **1**, the methyl ester of glucuronic acid tetraacetate, and the peracetylated methyl ester of the metabolite. The methyl esters of D-glucuronic acid and the metabolite were prepared by treatment with diazomethane. These methyl esters, as well as **1**, were acetylated overnight with acetic anhydride and *N*-acetylimidazole in pyridine at 50°C. Strong infrared absorption at 3311 cm^{-1} (C≡C–H stretch) was observed for **1** and the acetate of **1**, but not for the metabolite and D-glucuronic acid derivatives. The carbonyl stretch at 1750 cm^{-1} was noted for all but **1**. Weak absorbance in the 2100 cm^{-1} region (C≡C stretching) was observed for **1**, the acetate of **1**, and the metabolite derivative. These observations support the assignment of structure **3** to the metabolite for two reasons. First, the lack of absorption in the 3300 cm^{-1} region for the metabolite indicated the absence of a terminal hydrogen on the acetylenic group. Second, the strong absorption of ethchlorvynol acetate at

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3311 cm^{-1}, with no absorption in the 3600 cm^{-1} region, indicated acetylation of the alcohol and not of the acetylenic group.

The proton magnetic resonance spectrum (*13*) of the metabolite in D_2O was compared to that of **1** in a mixture of CD_3OD and D_2O (1:1). The ethyl and the vinyl chloride groups were intact, but the acetylenic proton was absent. The presence of a multiplet (4H), 3.1 to 3.8 ppm (δ), and a doublet (1H), 4.7 ppm, $J = 7.7$ Hz, in the metabolite spectrum suggested a sugar moiety in the β conformation, as is normally the case for naturally occurring glucuronides.

All of the spectral data support the assignment of structure **3** to the major metabolite. Furthermore, our inability to demonstrate hydrolysis of the metabolite with β-glucuronidase is consistent with the observation (*2*) that the *C*-glucuronides of phenylbutazone and sulfinpyrazone are not substrates for the enzyme.

The identification of three types of *C*-glucuronides in mammalian systems suggests the possibility that many unidentified polar metabolites that are not hydrolyzed by β-glucuronidase may, in fact, be products of *C*-glucuronidation. This raises interesting questions regarding the specificity of the enzymes responsible for glucuronidation and the mechanisms by which they function.

CRAIG R. ABOLIN
THOMAS N. TOZER
J. CYMERMAN CRAIG
LARRY D. GRUENKE

Departments of Pharmacy and *Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco 94143*

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6. The instrument was a Waters Associates liquid chromatograph; pump model M6000A, injector model U6K, and detector model 440, dual wavelength. Waters μBondapak C_{18} reverse-phase columns were used.
7. An AEI model MS-12 single-focusing mass spectrometer was used to obtain the electron impact spectra. A modified model MS-902 double-focusing mass spectrometer, equipped with a direct inlet system, was used for chemical ionization spectra. Reagent gas was isobutane at a pressure of 0.5 torr.
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11. Determination of the chemical ionization mass spectrum at high resolution gave an MH^+ ion *m/e* 623.2452, which corresponds to the formula $C_{26}H_{52}ClO_7Si_4$ (calculated *m/e*, 623.2478).
12. The infrared spectrometer used was the Nicolet, model 7000 P, with Ge-KBr beamsplitter and liquid nitrogen–cooled detector. Samples were run as dilute (0.3 to 0.5 mg/ml) solutions in spectral-grade carbon tetrachloride; a 1-cm pathlength cell with calcium fluoride windows was used.
13. Proton magnetic resonance spectra were obtained with a Varian XL-100 spectrometer provided through NIH grant 00892-1A1 from the Division of Research Resources.
14. This work was supported by grant GM 16496 and predoctoral training grant GM 17175 from the National Institute of General Medical Sciences. We thank Dr. Murray Goodman and Wayne Bechtel of the Department of Chemistry, University of California, San Diego, for their help in obtaining the infrared spectra and Abbott Laboratories for providing a sample of ethchlorvynol.

3 March 1980

Critical-Path Scheduling of Mental Processes in a Dual Task

Abstract. *Scheduling theory is used to extend the additive factors method of analyzing reaction times to tasks involving concurrent processing. Data indicate that mental processes in a double-stimulation experiment by Becker are executed in a critical-path network. A relationship predicted for the timing of the responses of the task is shown to hold.*

When a set of mental processes, such as perceiving and deciding, must be executed to perform a task, the brain must resolve the same problem as that faced by computer operating systems and industrial managers, namely, how to schedule the many activities demanding attention. The problem is treated in the theory of scheduling (*1*). If this theory applies to mental processes, certain predictions follow for reaction times.

The primary technique for analyzing reaction times is Sternberg's additive factors method (*2*). Its usefulness is limited, though, mainly to tasks in which all processing is sequential. But concurrent processing is likely and of much theoretical interest since Townsend (*3*) has shown that both kinds of processing often yield the same reaction time distributions. Tasks incorporating both kinds of processing can be analyzed if Sternberg's method is combined with ideas from scheduling theory.

With the additive factors method, two experimental factors that increase reaction time are manipulated. Suppose (i) all the processes of a task are executed in a sequence and (ii) each factor prolongs a different process. The effect on reaction time of prolonging both processes will be the sum of the effects of prolonging them individually. A violation of additivity has usually been interpreted in the framework of the method as indicating that (ii) is false. But nonadditivity might indicate instead that (i) is false, that processing is not entirely sequential. Nonadditivity turns out to be likely when separate processes are prolonged in a task involving concurrent processing. For such a task, of course, there is no reason to expect the principles of the additive factors method to apply without modification.

The modifications presented here are based on critical-path scheduling (*1*), which allows both sequential and concurrent processes to occur. The technique requires only that the processes be partially ordered, that is, that they can be represented by a directed acyclic network (Fig. 1).

As the critical-path method is ordinarily used, the network is given, and the time required to complete the task is to be calculated. The psychologist has the opposite problem of knowing the time required to complete the task under various conditions and wanting to reconstruct the unknown network. The key to constructing the network is to use the idea from the additive factors method of prolonging processes. The effects of such prolongations are surprisingly informative about the network.

In a double-stimulation experiment by Becker (*4*), two factors have additive effects, but there is evidence that the underlying network is more complicated than a simple sequence of processes. In the experiment, the digit 1 or 2 was presented visually, and, after an interval of either 90 or 190 msec, a tone of high or low frequency was presented. On each trial the subject pressed a button with one hand to indicate which digit occurred and another with the other hand to respond to the tone. In one condition the subject pressed the same button for either tone, and in another condition he chose one of two buttons to indicate which tone occurred.

Table 1 gives the increases in response times produced by changing the experimental conditions. Departing from custom, I express response times for both responses measured from the onset of the first stimulus, the digit. Response times in the condition with one tone response choice and 90 msec between stimulus onsets are baselines from which

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