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A STUDY OF DRUG INTERACTION BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY— SYNERGISM OF CHLORAL HYDRATE AND ETHANOL

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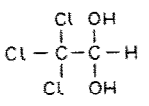
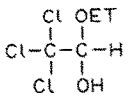
Abstract—During co-administration of chloral hydrate and ethanol-d_6 to a rat, 1-deutero-2,2,2-trichloroethanol was detected in the urine by means of gas chromatography–mass spectrometry (GC-MS). This confirms an earlier hypothesis that the synergistic effect of chloral hydrate and ethanol is due to their coupled redox reaction in the alcohol dehydrogenase-mediated enzymatic process.

The concurrent ingestion of chloral hydrate (I) and ethanol has long been known to produce a more pronounced central nervous system (CNS) depressant effect than that predicted by the simple additive potence of both components [1]. The name "knock-out drop" or "Mickey Finn" is well known for this drug combination [2]. This enhancement effect has been demonstrated in animals [3, 4], and clinical studies in man have been reported by Sellers *et al.* [5]. Early in 1937, Bastedo [6] suggested that the elevated potence was due to "chloral alcoholate" (II). The pharmacologic role of II was re-examined by Gessner and Cabana [7] who demonstrated that the hypnotic activity of II was, in fact, due to its dissociation into chloral hydrate and ethanol. Thus, the hypothesis of chloral alcoholate being the causative agent only presents a circular argument.



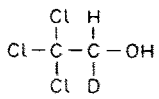
(I)



(II)

In general, the synergistic effect of one drug with another can be explained in terms of the interactions through alteration of distribution, absorption, biotransformation or excretion of the drugs involved [8]. Since chloral hydrate and ethanol are known to be metabolized by alcohol dehydrogenase in the presence of nicotinamide adenine dinucleotide (NAD) as co-enzyme [9], it is quite likely that the observed synergism is due to the interaction of these two drugs in the common metabolic pathway. Ethanol is oxidized to acetaldehyde by alcohol dehydrogenase coupled with reduction of NAD to reduced nicotinamide adenine dinucleotide (NADH). On the other hand, the major metabolic pathway of chloral hydrate involves its reduction to trichloroethanol in the presence of alcohol dehydrogenase and NADH as the hydrogen donor. Thus, it could be expected, as Cabana and Gessner [10] have already suggested, that the administration of ethanol and its subsequent oxidation to acetaldehyde could increase the rate of reduction of the simultaneously administered chloral hydrate to trichloroethanol. Since the latter is a more potent hypnotic agent than chloral hydrate [11], an enhanced CNS depression effect would be expected. Results from studies *in vitro* by Freeman and Schulman [12] were in agreement with this hypothesis. Using human liver dehydrogenase, these workers were able to demonstrate an enhancement in the coupled redox reaction for ethanol and chloral hydrate. Quantitative data have been reported by Sellers *et al.* [13] who studied the interaction of chloral hydrate and ethanol metabolism in human volunteers by measuring the levels of the parent drugs and their metabolites.

While these data indicate a close interrelationship of the oxidation of ethanol and reduction of chloral hydrate, no direct measure of the coupling of these two reactions *in vivo* has been reported. It appeared that this could be settled by a demonstration that the α-hydrogen atom lost in the oxidation of ethanol is indeed transferred to the chloral hydrate molecule when the latter is reduced to trichloroethanol. Therefore, we have designed the following experiment which demonstrates the formation of 1-deutero-2,2,2-trichloroethanol (III) upon the administration of a mixture of ethanol-d_6 and unlabeled chloral hydrate.



(III)

MATERIALS AND METHODS

System in vivo. Three 7-week-old Sprague–Dawley rats were kept separately in metabolism cages fitted with funnels to collect urine. A dose of 40 mg chloral hydrate in 1 ml water was administered orally to one rat. Another animal was given a dose consisting of 40 mg chloral hydrate and 1 g ethanol in 1 ml water. The remaining rat was given 40 mg chloral hydrate and 1 g ethanol-d_6 (Merck Chemicals, Quebec, Canada) in 1 ml water. Urine was collected con-

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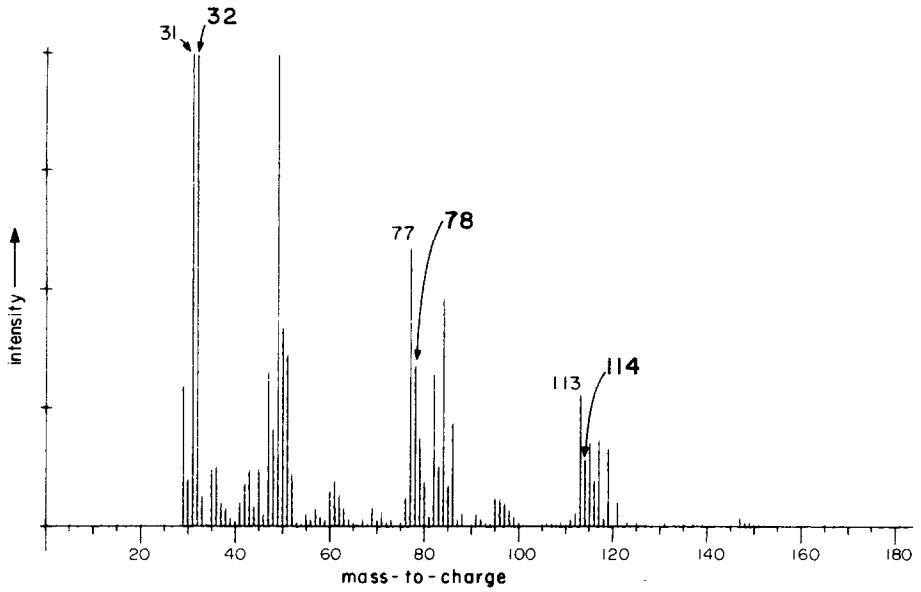


Fig. 1. Mass spectrum of the trichloroethanol metabolite obtained from the urine of the rat fed with chloral hydrate and ethanol-d_6. The ions outlined in Scheme I are marked; the light numbered ones are due to the unlabeled metabolite (IV), while the bold numbered ones are due to the labeled metabolite (III).

tinuously for 24 hr. An aliquot of 15 ml of each urine sample was extracted twice with 80 ml dichloromethane. The combined organic layer was dried with anhydrous sodium sulfate. It was then evaporated down to about 1 ml with a rotary evaporator, and further concentrated to about 15 μl with a stream of nitrogen. A 2-μl aliquot of this final extract was taken up by a syringe for gas chromatography–mass spectrometry (GC–MS) analysis.

Trimethylsilylation. To 3 μl of urine extract in a capillary tube was added 10 μl acetonitrile and 15 μl *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The capillary tube was sealed and warmed to 60° in a sand bath for 15 min. The seal was then broken and the mixture was concentrated in vacuum followed by GC–MS analysis.

Gas chromatography. A Perkin–Elmer 990 gas chromatograph was used, equipped with a 3 ft × 1/8 in.

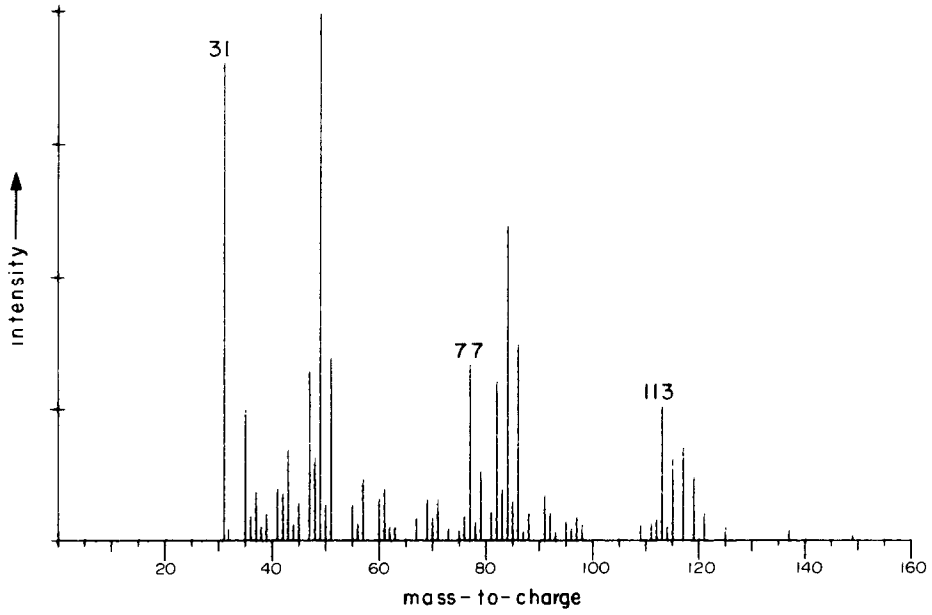


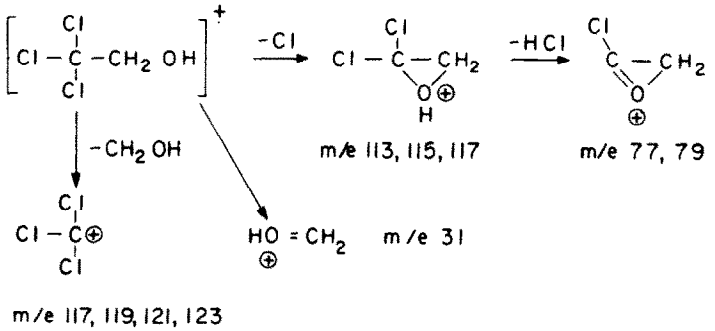
Fig. 2. Mass spectrum of the trichloroethanol metabolite obtained from the urine of the rat fed with chloral hydrate and ethanol. Note that the intensities of the ions at m/e 32, 78 and 114 are very small compared to those of Fig. 1.

glass column of 3% OV-17 on Gas-Chrom Q (100–120 mesh). It was temperature programmed from 60 to 200° at 16°/min.

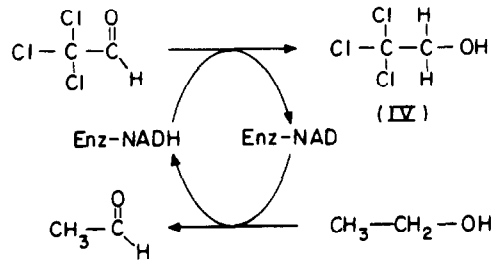
Mass spectrometry. An Hitachi Perkin–Elmer RMU-6L mass spectrometer was interfaced to the Perkin–Elmer 990 gas chromatograph via a fritted glass separator. An IBM 1800 computer was used for data acquisition and control [14, 15]. Mass spectra were recorded continuously during the entire gas chromatogram. The ionization voltage was 70 eV; the temperature of the ion source was 220° and that of the manifold was 250°.

RESULTS AND DISCUSSION

Each of the GC–MS analyses of the urine extracts from the three rats indicated the presence of trichloroethanol as expected. This metabolite was more abundant in the urine of the two animals to which ethanol or ethanol-d_6 was co-administered with chloral hydrate. Moreover, the mass spectra of this component obtained from these two experiments were quite different (Figs 1 and 2). The mass spectrum taken from the experiment in which the animal had ingested a combination of chloral hydrate and ethanol-d_6 clearly demonstrated the presence of a mixture of unlabeled trichloroethanol and trichloroethanol-d_1. Thus, in Fig. 1, ions at m/e 114, m/e 78 and their chlorine isotope clusters are indicative of incorporation of one deuterium atom into the molecule. The unlabeled molecule has the corresponding fragments at m/e 113 and m/e 77 respectively. The fragmentation pattern for the unlabeled compound is outlined in Scheme I. The presence of ion m/e 78 for the labeled compound would suggest that the deuterium atom was incorporated onto the alpha-carbon. The possibility of a deuterated hydroxyl group giving rise to such an ion, which might have arisen by a simple exchange of hydroxyl hydrogen for deuterium from ethanol-d_6, is unlikely due to the high dilution factor of ethanol-d_6 in the body water of the animal. Deuterium in any hydroxyl group would, in fact, be lost to the water. We further ruled out the possibility unambiguously by trimethylsilylation of the hydroxyl group. The mixture of labeled and unlabeled trichloroethanol obtained from the animal experiment upon trimethylsilylation gave a mass spectrum, as shown in Fig. 3. The M-15 ion of the unlabeled compound which arises by loss of a methyl moiety from the trimethylsilyl group has a mass-to-charge ratio of 205 and chlorine isotope ions at m/e 207, 209 and 211. The deuterated analogue has the corresponding ions at one mass unit higher. Thus, it is certain that the deuterium is incorporated at the alpha-carbon. This requires a transfer of deuterium from ethanol-d_6 to the aldehydic carbon of chloral hydrate. From the relative intensities of the ions of m/e 77 and m/e 78, and the ions of m/e 113 and m/e 114 in the mass spectra of trichloroethanol samples from the two experiments (Figs 1 and 2), one can conclude that about 30 per cent deuterium is incorporated under these experimental conditions when feeding deuterated ethanol. This high incorporation of deuterium into trichloroethanol is indicative of a well coupled redox reaction between chloral hydrate and ethanol. These experiments demonstrate that the hypothesis previously put forward by Cabana and Gessner [10] and by Sellers *et al.* [13] is correct. The enzymatic redox transformation of chloral hydrate and ethanol involves alcohol dehydrogenase, and it is known that this enzyme participates in redox processes as an enzyme–NAD or enzyme–NADH complex depending on the direction of the reaction. The rate-determining step of this enzymatic reaction is the dissociation of the enzyme–coenzyme complex [16, 17]. Thus, when chloral hydrate alone is administered to an animal, the rate of production of trichloroethanol is dependent on the dissociation of the enzyme–NAD complex for replenishment of free enzyme to combine with another molecule of NADH, which can then be available for further reduction of chloral hydrate to trichloroethanol. However, in the case of co-administration of chloral hydrate and ethanol, an enzyme–NAD complex used in oxidation of ethanol to acetaldehyde provides the necessary enzyme–NADH complex for reduction of chloral hydrate to trichloroethanol. This, in turn, rejuvenates the enzyme–NAD complex for further redox cycles (Scheme II). Thus, the rate of conversion of chloral hydrate to trichloroethanol is faster. A similar scheme has been postulated in a system of ethanol and lactaldehyde on the basis of kinetic studies [18, 19]. As the detoxification rate of trichloroethanol via glucuronide formation is unaffected by the addition of ethanol, an accumulation and hence higher concentration of trichloroethanol are observed in this experiment as well as in



Scheme I



Scheme II

others [10]. Since trichloroethanol is a more potent hypnotic agent than the parent drug, a synergistic effect results. The enhanced reduction of chloral hydrate to trichloroethanol also explains why less trichloroacetic acid, another metabolite of chloral hydrate, was found during co-administration experiments in earlier studies [4]. As suggested earlier [10], this is simply because the more rapid reduction of chloral hydrate makes the drug less available for transformation to trichloroacetic acid in the alternate metabolic pathway.

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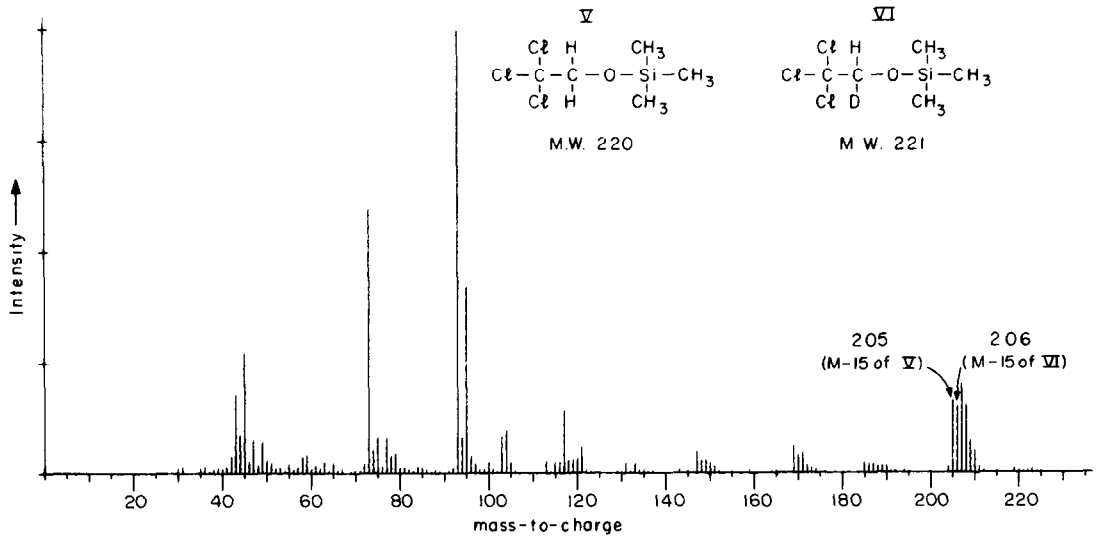

Fig. 3. Mass spectrum of the trimethylsilyl derivative of the trichloroethanol metabolite obtained from the urine of the rat fed with chloral hydrate and ethanol-d_6.

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