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REACTIONS OF TRICHLOROETHYLENE EPOXIDE IN AQUEOUS SYSTEMS

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Trichloroethylene (tri) is carcinogenic in long-term animal experimentation in mice but not in rats (1). Suspicion has been raised that the carcinogenic properties of tri might be due to the metabolic formation of an electrophilic epoxide which in turn might alkylate essential cellular macromolecules (2,3,4). This would be analogous to results obtained with the chemically similarly structured vinyl chloride. This hypothesis has been supported by studies of alkylating reactions of tri-epoxide with some low molecular model nucleophiles in non-polar solvents (5). Further evidence is provided by the demonstration of covalent binding of ^{14}C-tri metabolic conversion products in vitro (6) and in vivo by the action of liver microsomes (7). In addition, a specific P-450 binding spectrum in liver microsomes has been observed after addition of tri and tri-epoxide (8).

Objections to this concept have been raised by findings that the metabolites of tri could only be derived from trichloroacetaldehyde and not from the thermal rearrangement product of tri, dichloroacetic acid, nor from tri-epoxide itself (9, 10). An explanation has been provided for the apparently exclusive rearrangement observed in vivo. It has been demonstrated that tri-epoxide can be forced to rearrange completely to trichloroacetaldehyde by the catalytic action of Lewis acids, like $FeCl_3$ or $AlCl_3$ (11, 12). Such formation of a comparatively non-reactive product could be regarded a detoxication and a protective mechanism against a possible carcinogenic action of tri within the mammalian organism (13). To further elucidate the role of tri-epoxide in metabolism as well as in mutagenicity and carcinogenicity of tri, we have studied its reactivity in aqueous systems in order to approach physiological conditions encountered by tri-epoxide as it is formed in vivo.

Materials and Methods

Preparation of tri-epoxide. A sample of 600 ml tri (Merck Nr. 948, stabilized by triethanolamine) was continuously bubbled through with oxygen and irradiated in a UV-high pressure mercury submersion lamp for 6 hrs (14). The concomitantly formed dichloroacetylchloride was removed by washing with chilled 6 N NaOH. Unreacted tri was removed by distillation under reduced pressure. The residue was separated, by three subsequent steps, in a preparative gas chromatograph (30% squalan on chromosorb W/AW, 2 m, 4 mm inner diameter, 50°C); tri-epoxide was trapped under N_2 at -80°C and stored in small portions at -50°C.

Titration of acids. A solution of 10 mM tri-epoxide in distilled water was vigorously shaken for 5 min. Samples of 10 ml were titrated to neutral with 0.1 N KOH using a glass electrode.

Determination of chloride ions. The same solution was used for a Cl^--titration with $AgNO_3$ (0.1 N) according to Mohr.

Formic acid. The sample was acidified (H_2SO_4), steam distilled, reduced to formaldehyde by metallic magnesium in strong acid solution (25% HCl), and combined with chromotropic acid (0.25% solution in 81% H_2SO_4, 1 ml in total vol of 10 ml) according to Nash (14). The violet colour was measured at 578 nm against blanks.

Carbon monoxide. Liquid samples such as those used for the HCOOH-determination were transferred to air-tight tubes, and aliquots of the gas phase were injected into a GC-system consisting of 4 columns: a) 1 m carbowax 20 M at room temp. (retention of C_2-compounds), b) 30 cm KOH 10% on chromosorb P/AW at 80° (stripping of CO_2), c) 2 m molecular sieve 4 Å at 80° (separation of low molecular gases), d) approximately 5% Ni on chromosorb P/AW at 350° (reduction of CO to CH_4). Helium at a flow rate of 20 ml/min was used as carrier gas; a hydrogen flow of 30 ml/min was added between columns c and d for purpose of reduction. FID detection was optimized by an additional purge-flow of 20 ml/min N_2 to the FID.

A GC-system consisting of columns b-d has been referred to in the literature for analysis of CO-Hb (16).

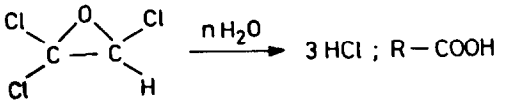
Glyoxylic acid. 10 µl tri-epoxide (100 µmoles) were transferred to 10 ml 1 N HCl. After vigorous shaking (5 min) and standing undisturbed for 1 hr the water was evaporated under reduced pressure at 30°C. The oily residue was injected into a Varian CH7 mass spectrometer.

Chloral, trichloroacetic acid and trichloroethanol. This was performed with well established GC-methods (17, 18).

Dichloroacetic acid was determined after esterification with gas chromatography (19).

Results and Discussion

1) Formation of acids. Surprisingly, trichloroethylene epoxide decomposes, in unbuffered water, completely into several acid products. The titration of the 10 mM solution, after completion of the reaction, results in 4 acid equivalents and 3 Cl^- per mole: in 4 experiments with 50 µl of tri-epoxide (D= 1.53) in 50 ml H_2O the required volumes per 10 ml of this solution were 4.06±0.01 ml 0.1 N KOH (theory 4.0) and 3.12±0.00 ml 0.1 N $AgNO_3$ (theory 3.0). Thus, all chlorine atoms of tri-epoxide are split off under the formation of hydrochloric acid. The one remaining acid equivalent should be expected to be (an) organic acid(s). A rough estimation of the mechanism and balance of tri-epoxide decomposition under the test conditions is as follows:



2) Formation of carbon monoxide and formate. Tri-epoxide decomposes, under C-C fission, to CO and HCOOH. The proportion of C_1-units formed, in respect to the overall rate as well as to the single compounds, is clearly dependent on the pH (Table 1). In strong alkali, the molecule splits almost completely into equal parts of CO and HCOOH, whereas the yield of these compounds steadily decreases with falling pH. Under strong acid conditions (pure hydrochloric acid) the formation of these C_1-units amounts to only 10%. The experiments with unbuffered water fit well in the series if the pH value of 1.5 at the end of reaction is taken as representative for the reaction conditions.

Since there is a significant negative shift in the formation of CO and HCOOH with decreasing pH, other decomposition products should also be expected. Careful GC analysis reveals the complete absence of tri chlorinated C_1 or C_2 molecules, e.g. chloral, trichloroethanol, or trichloroacetic acid. This has led us to search for other decomposition products.

Tab. 1: Formation of carbon monoxide, formate and dichloroacetic acid from trichloroethylene epoxide under varying pH in aqueous systems. Incubation mixture: 100 µM in 10 ml solution, vigorously shaken, left overnight at 4 °C. n=number of determinations

Medium	M	pH start	pH end	products identified (% theory of C_2HCl_3O) CO	HCOOH	n	$CHCl_2$-COOH	n
H_2O	-		1.8	16.0±1.3	16.5±2.1	5	3.4±1.3	3
KOH	0.1	13.0	12.8	47.8±9.3	46.5±2.4	6	6.25±1.7	3
NaOH	1.0	14.0	13.6	49.0±9.6	46.9±4.1	7	15.3±2.4	3
Tris-HCl	0.5	9.0	8.9	35.3±4.9	17.9±3.2	3	13.8±1.7	3
Tris-HCl	0.5	7.4	7.2	27.7±2.0	15.2±1.1	8	24.8±1.7	3
HCl	0.1	1.0	1.1	14.8±1.1	12.5±1.6	4	13.5±2.4	3
HCl	1.0	0	0.2	6.3±0.75	6.9±1.7	4	29.4±5.7	3

3) Formation of glyoxylic acid. Mass spectrometry of samples of tri-epoxide hydrolysate prepared as described above reveals the presence of glyoxylic acid (Table 2).

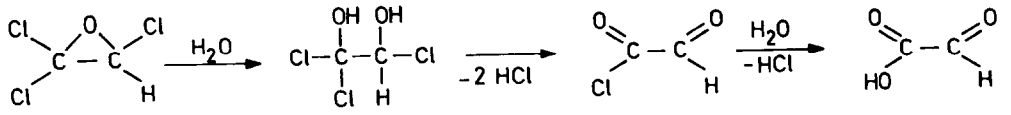
Tab. 2: Mass spectrometric identification of glyoxylic acid

Fragments:	$C_2H_2O_3$	C_2O_2	CH_2O_2	CHO_2	CO_2	CH_2O	CHO	CO
M/e	74	56	46	45	44	30	29	28
Intensity(%)	5.1	30.7	53.8	64.1	46.2	66.7	100.0	53.8

The molecular peak of glyoxylic acid (M=74) is distinctly detectable at 5%. The seven fragments correspond well to theoretical expectations. Comparison between the mass spectra of a.r. glyoxylic acid and the tri-oxirane hydrolysate reveals identity. The amount formed is calculated to be very low, quantitative determinations have not yet been performed.

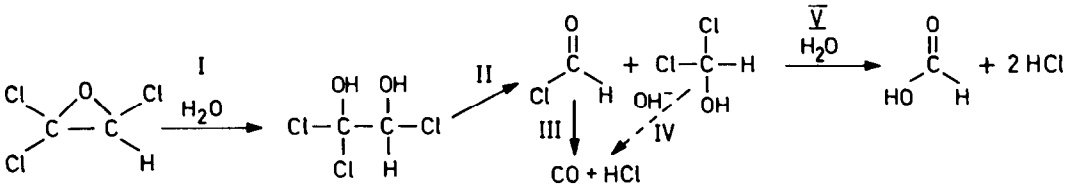
4) Formation of dichloroacetic acid (di). The thermal rearrangement product of tri-oxide, dichloroacetylchloride or its hydrolysis product (di), resp. is formed in varying amounts (Table 1). It is lowest in pure H_2O and highest at pH 7.4 as well as in strong acid solution. The inconsistency in the experiment with NaOH is unexplained at present; the amount of di formed seems to depend on the velocity of preparing and shaking of the reaction mixture.

5) Possible mechanism of tri-epoxide degradation. The formation of glyoxylic acid from trichloroethylene epoxide can plausibly be explained by the assumption of a hydrolytic opening of the oxirane ring under formation of the diol. Subsequent elimination of one HCl from each involved carbon atom should be expected on account of the instability of geminal substitution by Cl and OH which tends to carbonyl formation:



The resulting glyoxylic acid chloride will, in a final step, hydrolyse to the free acid.

The formation of carbon monoxide and formate may be formulated as follows:



The second step (II) would be a C-C fission under the migration of one proton. The decomposition of formyl chloride (III) and dichlorohydroxymethane (IV), an intermediate in methylene chloride metabolism (20), are well established reactions. The pH-dependent shifts in Cl^- and $HCOO^-$ formations, as well as the (limited) rearrangement of tri-epoxide to dichloroacetyl chloride are consistent with all assumed reactions. Also, the increase of CO formation with increasing pH can be explained by the concurrence of steps III and IV.

Conclusions

The results clearly demonstrate that tri-epoxide decomposes rapidly, under physiological conditions, by means of C-C and C-Cl fissions, predominantly to C_1-units. Contrary to this, tri is metabolically transformed *in vivo* exclusively to trichlorinated C_2-compounds (21), without any C-Cl fission (22). In addition, microsomal tri-oxidation does not result in CO-formation (23). These facts do not support, at first glance, epoxidation as a metabolic pathway. However, we have demonstrated previously (10) that tri-epoxide rearranges, contrary to expectation from its thermal rearranging behaviour, to chloral in the presence of Lewis acids. Accordingly, we can offer the following explanation for the above outlined discrepancy: tri is epoxidised by mixed function oxygenases; tri-epoxide rearranges immediately within the hydrophobic premise of the enzyme by virtue of the catalytic action of the trivalent iron of P450 to the nonreactive chloral so that, under normal *in vivo* conditions, the highly reactive epoxide is protected from the decomposition reactions as described in this communication. This hypothesis would be consistent with the very low mutagenic (2) and doubtful carcinogenic (13) potential of tri. A confirmation is expected in forthcoming experiments which also have to clarify the type,

fate and significance of the relatively low amount of covalent binding of metabolized tri as described by others (5, 6).

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REFERENCES

1. NCI-Technical Report Series No. 2, Feb. 1976 (Washington)
2. H.Greim, G.Bonse, D.Reichert, D.Henschler, Biochem.Pharmacol. 24, 2013 (1975)
3. B.L.van Duuren, Ann.N.Y.Acad.Sci. 246, 258 (1975)
4. B.L.van Duuren, Environm.Hlth Perspect. 21, 17 (1977)
5. S.A.Kline, B.L.van Duuren, J.Heterocyclic Chem. 14, 455 (1977)
6. H.Uehleke, S.Poplawski-Tabarelli, Arch.Toxicol. 37, 289 (1977)
7. H.Uehleke, Th.Werner, Arch. Toxicol. 34, 289 (1975)
8. H.Uehleke, S.Tabarelli-Poplawski, G.Bonse, D.Henschler, Arch. Toxicol. 37, 95 (1977)
9. G.Bonse, Th.Urban, D.Reichert, D.Henschler, Biochem. Pharmacol. 24, 1829 (1975)
10. G.Bonse, D.Henschler, Crit.Rev.Toxicol. 4, 395 (1976)
11. D.Henschler, Environm.Hlth Perspect. 21, 61 (1977)
12. D.Henschler, G.Bonse, 7th Int.Congr.Pharmacol.Abstr. 270, Paris, July 16-21, 1978
13. D.Henschler, E.Eder, T.Neudecker, M.Metzler, Arch.Toxicol. 37, 233 (1977)
14. F.Österreicher, Thesis, Univ. Wien 1967
15. T.Nash, Biochem.J. 55, 415 (1953)
16. J.Angerer, in D.Henschler (Ed.): Analysen in biologischem Material, Bd. 2, Verlag Chemie, Weinheim 1978
17. G.Müller, M.Spassovski, D.Henschler, Arch.Toxicol. 29, 335 (1972)
18. A.Eben, in D.Henschler (Ed.): Analysen in biologischem Material, Bd. 2, Verlag Chemie, Weinheim 1978
19. A.C.Monster, G.Boersma, Int.Arch.Occup.Environm.Hlth 35, 155 (1975)
20. V.L.Kubic, M.W.Anders, R.R.Engel, C.H.Barlow, W.S.Caughey, Drug Metab. Dispos. 2, 53 (1974)
21. G.Müller, M.Spassovski, D.Henschler, Arch.Toxicol. 32, 283 (1974)
22. J.W.Daniel, Biochem.Pharmacol. 12, 795 (1963)
23. H.Fetz, W.R.Hoos, D.Henschler, Naunyn-Schmiedeberg's Arch.Pharmacol. 302, Suppl.Abstr. 88 (1978)