THE INADEQUACY OF THE RANEY NICKEL DESULFURATION METHOD FOR THE STRUCTURAL ELUCIDATION OF AN EPOXIDE-GLUTATHIONE *S*-CONJUGATE: DESULFURATION OF PHENYLOXIRAN-GLUTATHIONE *S*-CONJUGATES TO PHENYLOXIRAN

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Both of purely separated regioisomers of phenyloxiran-glutathione *S*-conjugates, conjugate **1** (*S*-(1-phenyl-2-hydroxyethyl)glutathione) and conjugate **2** (*S*-(2-phenyl-2-hydroxyethyl)glutathione), afforded phenyloxiran as a common intermediate to the major desulfuration products, phenethyl alcohol and ethylbenzene, and to the minor ones, methylphenylcarbinol and toluene, on the mild treatment with Raney nickel in boiling ethanol. Phenyloxiran formed was hydrogenated to stable phenethyl alcohol and unstable methylphenylcarbinol under the catalytic conditions used, the latter of which was readily deoxygenated to ethylbenzene. The present study indicated that the Raney nickel method, despite of its being recognized to be well established, could not be applicable to the structural assignment of epoxide-glutathione or epoxide-mercapturic acid conjugates.

Keywords — epoxides-glutathione *S*-conjugates; phenyloxiran; *S*-(1-phenyl-2-hydroxyethyl) glutathione; *S*-(2-phenyl-2-hydroxyethyl) glutathione; phenethyl alcohol; methylphenylcarbinol; ethylbenzene; toluene; Raney nickel; desulfuration

Glutathione (GSH) *S*-conjugation by GSH *S*-transferase is of importance in view of not only detoxication of epoxides of a wide variety of exogenous olefins and arenes with carcinogenicity or toxicity,¹⁾ but also activation of leukotriene A formed from arachidonic acid *via* its hydroperoxide, 5-HPETE, to SRS (slow reacting substance of anaphylaxis)-A in murine mast cell tumor.²⁻⁷⁾ Structures and homogeneity of most of the xenobiotic conjugates have not been well studied yet except several cases. They are only the conjugates of dihydronaphthalene oxide,⁸⁾ benzo[*a*] pyrene 4,5-oxide,⁹⁾ phenyloxiran,¹⁰⁻¹²⁾ and naphthalene oxide.¹³⁾ Hydrogenolytic desulfuration using Raney nickel has been recognized to be a useful method for the elucidation of the structure of epoxide-glutathione *S*-conjugates, *e.g.* the conjugates of dihydronaphthalene oxide¹⁴⁾ and phenyloxiran.¹¹⁾

In the previous communication, the authors have demonstrated the inadequacy of the application of the Raney nickel method to the assignment of the sulfur-bearing carbon atoms of regioisomeric GSH conjugates of phenyloxiran.¹²⁾ The present paper deals with the formation of phenyloxiran as a common intermediate from the regioisomers of phenyloxiran-glutathione conjugates by Raney nickel and with hydrogenolysis and deoxygenation of the epoxide in the reaction medium.

MATERIALS AND METHODS

Materials — Ethylbenzene, methylphenylcarbinol, phenyloxiran, and Raney nickel were purchased from Wako Pure Chemical Industries, Ltd., Osaka. The epoxide was redistilled under reduced pressure before use. The nickel reagent was treated by the previous method also before

use to adjust it as a catalyst with the grade W-1 activity.¹⁵⁾ The treated catalyst was suspended in absolute ethanol and left stand for 1 h to obtain a fine suspension by decantation. Conjugate 1 (*S*-(1-phenyl-2-hydroxyethyl)glutathione) and conjugate 2 (*S*-(2-phenyl-2-hydroxyethyl)glutathione) were prepared as previously reported.¹²⁾

Raney Nickel Reaction — The catalyst suspension was heated at 79°C in a flask with a reflux condensor after the addition of a solution of the substrate(s) in absolute alcohol. For the termination of the reaction, the mixture was diluted with ether (4 vol.) and shaken with water (0.5 vol.). The organic layer separated by centrifugation was taken for subsequent gas-

chromatograph-mass spectrometer (GLC-MS) analysis.

GLC-MS — Mass spectra were recorded on a Shimadzu-LKB Model 9000 GLC-MS equipped with a column packed with 15% diethylene glycol succinate coated on 60–80 mesh Chromosorb W, 3 mm × 2 m. The flow rate of helium as carrier gas was 20 ml/min. Temperatures for the injection port, separator, and ion source chamber were kept at 250°C, 280°C, and 310°C, respectively. The ionization energy applied was 20 eV.

RESULTS AND DISCUSSION

Synthetic regioisomeric glutathione conjugates 1 and 2 (Chart 1), which had proved to be identical with the metabolites of racemic phenyloxiran by rat liver cytosolic glutathione *S*-transferase,¹²⁾ were heated under very mildly refluxing conditions with a small amount of Raney nickel (1.2 mg/ml), the activity of which was adjusted adequately so that the reaction profile could be ready to follow. GLC-MS analysis of the products after the extraction with ether to remove the catalyst and the water-soluble substrates indicated that, at 60 min after the reaction, phenethyl alcohol and phenyloxiran were the major products, and methylphenylcarbinol and ethylbenzene as the minor ones from both conjugates 1 and 2 (Table I). The products analyzed by GLC-MS showed molecular ion peaks at corresponding *m/e* values and characteristic fragment ion peaks identical with those from the authentic specimens: retention time (*t_R*) at 5.9 min, *m/e* 120 (*M*⁺), 92, and 91 (base peak) for phenyloxiran; *t_R* 14.6 min, *m/e* 122 (*M*⁺), 92, and 91 (base peak) for phenethyl alcohol; *t_R* 9.6 min, *m/e* 122 (*M*⁺), 107 (base peak), 79, and 43 for methylphenylcarbinol; and *t_R* 4.1 min, *m/e* 106 (*M*⁺), 92, and 91 (base peak) for ethylbenzene. Phenyloxiran was the most predominant product yielded from both conjugates at any intervals checked throughout the reactions (Table I). Next to this, phenethyl alcohol was rather a major product from conjugate 2 as well as from conjugate 1. Methylphenylcarbinol was

CHART 1. Structures of Phenyloxiran-glutathione *S*-Conjugates

They were resolved and purely isolated on an octadecylsilicone column by HPLC and recrystallized from methanol. Structural identification has been made by NMR spectroscopy as previously reported.¹²⁾

always a minor product from both conjugates. The yield of the deoxygenated product, ethylbenzene, was higher in conjugate 2 than in con-

jugate 1.

However, only trace amounts of phenyloxiran were detected from the conjugates at 30 min

TABLE I. A Time Course Study of Desulfuration Reactions of Phenyloxiran-Glutathione S-Conjugates with Raney Nickel

Substrate (10 mM)	Reaction time (min)	Products formed ($\times 10^{-2}$ $\mu\text{mol/ml}$)
		Conjugate 1	10	15.6	41.0	0.9	0.0
	30	54.2	98.5	2.8	7.0
	60	213.0	340.0	2.3	15.0
Conjugate 2	10	3.2	37.0	0.7	23.0
	30	11.3	117.0	1.1	23.0
	60	145.5	420.0	5.0	54.0

The conjugates were gently refluxed with a Raney nickel preparation (1.2 mg/ml, W-1 grade activity) in absolute ethanol. The products were identified and determined by GLC-MS: retention times of ethylbenzene (at a column temperature of 60°C), phenyloxiran, methylphenylcarbinol, and phenethyl alcohol (at 120°C each) were 4.1, 5.9, 9.6, and 14.6 min, respectively, under the conditions as described in the text.

TABLE II. A Time Course Study of Reactions of Oxygenated Ethylbenzenes with Raney Nickel

Substrate (10 mM)	Reaction time (min)	Products formed ($\times 10^{-2}$ $\mu\text{mol/ml}$)
		10	n.s.	4	22	—
	30	27	4	67	—
	60	123	3	123	—
10	15	—	0.2	10
	30	39	—	0.5	6
	60	230	—	0.8	4
10	7	0	—	0
	30	11	0	—	0
	60	24	0	—	0

The oxygenated ethylbenzenes were treated with Raney nickel under the same conditions as described in Table I.

n.s. represents detectable but unmeasurable amounts ($< 0.05 \times 10^{-2}$ $\mu\text{mol/ml}$).

after the reaction when a larger amount of the nickel catalyst (6 mg/ml) was used under the same reaction conditions as mentioned above. From these reaction mixtures were detected phe-

nethyl alcohol and ethylbenzene as major desulfurated products and methylphenylcarbinol and toluene as minor ones. This suggests that phenyloxiran would be an intermediate of ethylbenzene

TABLE III. *Stability of Phenethyl Alcohol and Methylphenylcarbinol in the Raney Nickel Reaction medium*

Time (min)	Remaining substrates (%)		
	<chem>Ph-CH2-CH2-OH</chem>	<chem>Ph-CH(OH)-CH3</chem>	<chem>Ph-CH2-CH2-OH</chem> / <chem>Ph-CH(OH)-CH3</chem>
0	100	100	1.0
10	98.1	22.1	4.4
30	92.0	7.0	13.1
60	85.2	n.d.	∞

Equimolar phenethyl alcohol and methylphenylcarbinol (5 mM each) were treated with Raney nickel (4.5 mg/ml) under the same conditions as described in Table I. n.d. < 0.1 %.

CHART 2. *Reactions of Glutathione Conjugates of Phenyloxiran with Raney Nickel in Refluxing Absolute Alcohol*

and its oxygenated derivatives.

Phenylloxiran, methylphenylcarbinol, and phenethyl alcohol were used as the substrates for the reaction in the presence of a small amount of the catalyst (1.2 mg/ml) in order to confirm the intermediacy of the epoxide. A comparable time-course study of the reactions indicated all of the oxygenated ethylbenzenes to be precursors of ethylbenzene in order of methylphenylcarbinol > phenylloxiran > phenethyl alcohol at the final stage of the reactions (Table II). Phenylloxiran as the substrate yielded both methylphenylcarbinol and phenethyl alcohol, the latter of which, however, was preferentially formed. Methylphenylcarbinol, the best precursor of ethylbenzene, afforded small amounts of phenylloxiran and phenethyl alcohol, suggesting the presence of some molecular rearrangement mechanism in the conversion of the carbinol to these products. Phenethyl alcohol did not afford any of other oxygenated ethylbenzene and was likely to be the most stable of the three substrates. Toluene was detected in minor ratios from the reaction mixture containing either phenylloxiran or phenethyl alcohol, but not from methylphenylcarbinol. Phenethyl alcohol was a better substrate for toluene than phenylloxiran.

A direct comparison of the stability of phenethyl alcohol and methylphenylcarbinol to the nickel catalyst was made by using an equimolar mixture of the alcohols and an appropriate amount of the catalyst (4.5 mg/ml) to accelerate the reactions. Methylphenylcarbinol disappeared much faster than the counterpart from the reaction mixture (Table III).

Data, therefore, strongly suggest that the Raney nickel desulfuration method can not be used for the structural elucidation of phenylloxiran-glutathione conjugate(s) although the previous authors¹¹⁾ have claimed the biological formation of the regioisomers mainly based on this method with their chromatographically inseparable conjugates. As summarized in Chart 2, the present investigation indicates that phenethyl alcohol and methylphenylcarbinol are not necessarily direct desulfuration products from

the corresponding conjugates. Phenylloxiran is rather the major desulfuration product from both conjugates, which is then readily hydrogenated to the stable alcohol, phenethyl alcohol, and to the unstable one, methylphenylcarbinol. The carbinol has a brief half-life on the nickel catalyst because of the ready deoxygenation to ethylbenzene.

A treatment of phenylloxiran with Raney nickel in atmospheric hydrogen has been reported to yield phenethyl alcohol as the major product.¹⁶⁾ Deoxygenation of 2-alkyl-2-phenylloxirans to alkylphenylethanes has also been demonstrated under the same conditions.¹⁷⁾ Indene oxide yields the corresponding nonbenzylic alcohol specifically on Raney nickel in hydrogen as a gaseous phase.¹⁸⁾

The present investigation would provide a fundamental question as to the result of the historically famous study on the regiospecific conjugation of dihydronaphthalene oxide with glutathione¹⁴⁾ or *N*-acetylcysteine⁸⁾ in the rat since the reported regiospecific conjugation with its benzylic oxiran carbon has been based only on the identification of a nonbenzylic alcohol formed from the conjugate by the Raney nickel method under very similar conditions to those used in the present investigation.

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