

# Determination of the Oil/Water Distribution Coefficients of Glyceryl Trinitrate and Two Similar Nitrate Esters

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The oil/water distribution coefficients of glyceryl trinitrate, chloroglyceryl dinitrate (1-chloro-2,3-propanediol dinitrate), and ethylglyceryl trinitrate (1,2,3-pentanetriol trinitrate) are determined. The analytical method employed (described in detail) is based on the reduction of a portion of the nitrate of each ester to the nitrite upon alkaline hydrolysis. This was determined by diazotization of sulfanilic acid and subsequent coupling with *N*-(1-naphthyl)-ethylenediamine and measuring the color spectrophotometrically.

IN CONNECTION WITH certain pharmacologic studies being conducted in this laboratory, information was desired concerning the oil/water distribution coefficient of glyceryl trinitrate and two closely related compounds of similar structure, *viz.*, chloroglyceryl dinitrate (1-chloro-2,3-propanediol dinitrate) and ethylglyceryl trinitrate (1,2,3-pentanetriol trinitrate). Since the water solubility of these substances is very low, the problem was to develop a method of estimation applicable to dilute aqueous solutions.

Under similar conditions, numerous workers have employed (in various modifications) the well known procedure of alkaline hydrolysis of glyceryl trinitrate resulting in the formation of nitrite which can be determined spectrophotometrically with a high degree of sensitivity through the customary diazotization and coupling procedure. We found this general method of analysis for glyceryl trinitrate, used successfully by Frauch and Burgin (1), applicable to the other two nitrate esters. This communication describes the method we have developed and its application to the determination of the desired oil/water distribution coefficients.

## EXPERIMENTAL

**Materials.**—The three nitrate esters used for study were prepared in this laboratory.

1-Chloro-2,3-propanediol dinitrate was prepared according to the method of Henry (2). It assayed 99.2%.

Glyceryl trinitrate was prepared by nitration of C.P. glycerin with mixed sulfuric-nitric acids (3). It was purified by repeated washings with water and very dilute sodium bicarbonate solution to remove soluble impurities and traces of nitration acids, then dried over anhydrous sodium sulfate. It assayed 99.0%.

1,2,3-Pentanetriol trinitrate was obtained by the nitration of 1,2,3-pentanetriol ("ethyl glycerine")

with a mixture of equal volumes of red fuming nitric (d. 1.60) and concentrated sulfuric acid at  $-10$  to  $0^{\circ}$ . One-hundred and fifty milliliters of mixed acid was used to nitrate 20 ml. of the triol. The crude nitrate, obtained as a blue oil upon pouring the nitration mixture into 1 L. of finely crushed ice, was purified by repeated washings with 500-ml. portions of ice water until the oil was almost colorless and the washings nearly neutral. The purified oil was dried over anhydrous sodium sulfate. Yield: 8.6 Gm.  $d_{20}^{20}$  1.4377,  $n_D^{20}$  1.4570. It is miscible with alcohol and acetone in all proportions and very slightly soluble in water. Analyses, adapting the U.S.P. XV-Devarda's alloy reduction procedure for glyceryl trinitrate, indicated a purity of 101.5%.

**Reagents and Apparatus.**—Wherever possible reagent grade chemicals were used. The following aqueous solutions are required: sodium hydroxide (2 *N*), hydrochloric acid (4 *N*), sulfanilic acid (2 mg./ml.), and *N*-(1-naphthyl)-ethylenediamine dihydrochloride (1 mg./ml.). This latter solution was found to be sufficiently stable over a period of some weeks when stored in an amber glass bottle. The oil used for the distribution experiments was Wesson corn oil.

The complete reaction, including hydrolysis and subsequent diazotization, was carried out conveniently in 15-ml. graduated glass-stoppered Pyrex centrifuge tubes. The colorimetric determinations were made with a Beckman model DU spectrophotometer using absorption cells having a light path of 1 cm. An efficient shaking device is required which can be regulated to avoid the formation of stubborn oil/water emulsions.

**Procedure.**—Standard solutions of each of the three esters, which are required for the establishment of standard curves, were prepared in the following manner: approximately 50 mg. of the nitrate ester was accurately weighed into a 50-ml. volumetric flask. One milliliter of absolute methanol was added and thoroughly mixed with the ester. Forty milliliters of warm water was added, immediately followed by vigorous shaking until the solution was complete. After cooling to room temperature, water was added to the mark followed by thorough mixing. These standard solutions were diluted quantitatively for use. A concentration of 5 mcg./ml. is satisfactory.

Triplicate aliquots of this diluted standard of 0.5, 1.0, 1.5, 2.0, and 2.5 ml. were transferred to the graduated centrifuge tubes; water was added to the

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4-ml. mark. Three tubes containing 4 ml. of water served as controls. After the addition of 1 ml. of 2 *N* sodium hydroxide, mixing was accomplished by the inversion of the glass-stoppered tube. After the removal of the stoppers, the tubes were placed in a boiling water bath. It is important at this step to employ a rack of sufficient size to hold all the tubes of a given experiment so that they can be immersed in the boiling water and removed simultaneously. After a period of 10 minutes in the boiling water, the tubes are removed and (after cooling to room temperature) the contents of each tube were treated with the following reagents, respectively, (with mixing after each addition by inversion of the glass-stoppered tube): (a) 1 ml. 4 *N* hydrochloric acid, (b) 1 ml. sulfanilic acid solution, (c) 1 ml. *N*-(1-naphthyl)-ethylenediamine solution plus water to the 10-ml. mark. After standing 10 minutes, the absorbance of the solution in each tube was measured at 548  $m\mu$  with a slit width of approximately 0.03 mm. Distilled water was used as the reference standard. The corrected triplicate absorbance values were averaged and plotted against the concentration. Typical standard recovery curves for the three esters are shown in Fig. 1. A linear relation is apparent for each ester and the reproducibility was satisfactory.

For the determination of the oil/water distribution coefficients, the following procedure was carried out for each of the nitrate esters: approximately 25 ml. of corn oil was transferred to a tared 250-ml. glass-stoppered Erlenmeyer flask and accurately weighed. A second accurate weighing was made after the addition of a suitable amount of the ester (0.1 to 0.3 Gm.). After gentle rotation of the flask for some seconds, exactly 25 ml. of distilled water was added. A similar flask containing 25 ml. of corn oil and 25 ml. of distilled water served as a control. The flasks were stoppered and shaken for 1 hour at room temperature. A good separation of the two phases was obtained after standing 15 minutes. Then a portion of the aqueous layer was removed and passed through No. 2 Whatman filter paper. Aliquots of 0.1 or 0.2 ml. of these filtrates were then analyzed by the same procedure employed for the preparation of the standard curves. A summary of the analytical data is shown in Table I. The final values obtained for the oil/water distribution coefficients are:  $109 \pm 3$  for glyceryl trinitrate,  $146 \pm 3$  for chloroglyceryl dinitrate, and  $97 \pm 2$  for ethylglyceryl trinitrate.

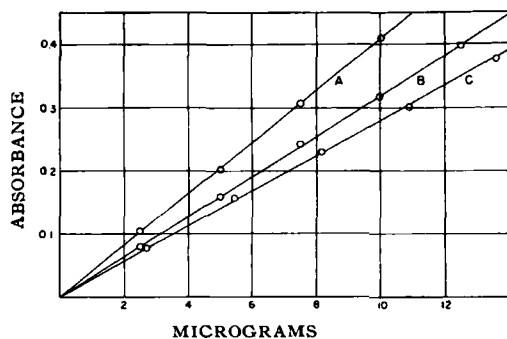


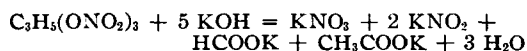
Fig. 1.—Typical standard recovery curves for the three esters: A, glyceryl trinitrate; B, chloroglyceryl dinitrate; C, ethylglyceryl trinitrate.

TABLE I.—SUMMARY OF EXPERIMENTAL DATA FOR THE DETERMINATION OF OIL/WATER DISTRIBUTION COEFFICIENTS

Ester	Expt.	Total Ester, Gm.	Ester in H <sub>2</sub> O Layer, mg.	Dist. Coeff. Oil/H <sub>2</sub> O
Glyceryl trinitrate	1	0.1676	1.70	110
	2	0.2267	2.33	110
	3	0.2829	2.84	108
	4	0.1191	1.25	106
	5	0.2412	2.50	112
	6	0.1619	1.65	109
				Av. $109 \pm 3$
Chloroglyceryl dinitrate	1	0.1266	0.95	147
	2	0.2015	1.51	149
	3	0.2530	1.90	148
	4	0.1690	1.32	143
	5	0.2249	1.72	145
	6	0.2338	1.85	143
				Av. $146 \pm 3$
Ethylglyceryl trinitrate	1	0.1778	2.00	98
	2	0.1057	1.25	95
	3	0.1419	1.55	98
				Av. $97 \pm 2$

## DISCUSSION

According to Hay (4), the saponification of glyceryl trinitrate is represented by



indicating a stoichiometry of reaction not supported by analytical data. According to Frauch and Burgin (1) the ratio of 2 moles of nitrite per mole of ester is usually lower and varies with the experimental conditions. The decomposition of nitrate esters is undoubtedly more complex, involving competition between hydrolytic reactions yielding nitrate, and internal oxidation-reduction (5) accounting for the formation of reduction products (nitrite and hydroxylamine) (6) and various oxidation products indicated in Hay's equation.

In this connection we have replotted the curves from Fig. 1 on a molar basis (as shown in Fig. 2) and have included for comparison a standard curve for sodium nitrite prepared from a standard solution of a reagent grade of this salt. It may be seen that the base catalyzed reaction of glyceryl trinitrate by

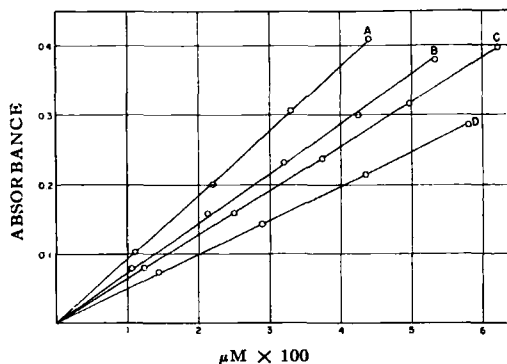


Fig. 2.—Typical standard recovery curves plotted on a molar basis: A, glyceryl trinitrate; B, ethylglyceryl trinitrate; C, chloroglyceryl dinitrate; D, sodium nitrite.

our procedure yields a nitrite:ester ratio appreciably lower than the value predicted by Hay.

If we apply the same general considerations to reactions involving the chloroglyceryl dinitrate and ethylglyceryl trinitrate and calculate ratios, we obtain an appreciably lower value in nitrite yield in the case of ethylglyceryl trinitrate. This suggests that the presence of an ethyl group has increased the stability of the molecule to oxidation-reduction or has favored reactions not resulting in nitrite formation. In the case of chloroglyceryl dinitrate having no nitrate group on carbon-1, an even higher degree of stability is indicated.

It appears that in each of the three nitrate esters one nitrate group is readily reduced and a second nitrate is reduced more slowly (or less completely).

In spite of this apparent lack of stoichiometry, the highly consistent results obtained from repeated trials indicate that with reasonable care in the control of experimental conditions employing standard recovery curves, a satisfactory analytical procedure can be devised.

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## Investigation of the Stereochemistry of Cycloheximide and Its Degradation Products

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Cycloheximide tosylate has been shown to undergo elimination of *p*-toluenesulfonic acid to give a new anhydrocycloheximide. By a series of reactions this new anhydrocycloheximide has been converted into a dideoxycycloheximide which was shown to be optically inactive. This observation establishes the *cis*-orientation of the methyl groups in the new dideoxycycloheximide. The series of reactions employed and the stereochemistry of the products obtained are discussed.

THE DETERMINATION of the stereochemistry of the glutarimide antibiotics has been under investigation recently in a number of laboratories (1-4, 7). On the basis of these studies, it has been demonstrated both by chemical degradation (7) and by thermal degradation (1) that the two methyl groups in cycloheximide are oriented in a *trans* manner. We have now observed certain other degradative and isomerization reactions on cycloheximide which bear on its stereochemistry.

Treatment of cycloheximide (I) with *p*-toluenesulfonyl chloride in pyridine gave a good yield of the corresponding tosylate (II). When an attempt was made to displace the tosylate group in II with iodide ion using acetone as the solvent, only unchanged starting material was recovered. Therefore, II was treated with sodium iodide in dimethylformamide; the product isolated from this reaction was not the corresponding iodide, but rather an iodine-free compound which exhibited ultraviolet absorption at 235 m $\mu$  ( $\epsilon$ , 9480). On the basis of its elemental analysis,

infrared and ultraviolet spectra, and its further reactions, this compound has been shown to be *epi*-anhydrocycloheximide (III).<sup>1</sup> Subsequently, it was learned that this interesting elimination and rearrangement reaction occurred merely by heating II in dimethylformamide. However, if the reaction is carried out in the presence of one equivalent of sodium bicarbonate, elimination of *p*-toluenesulfonic acid occurs with the formation of anhydrocycloheximide (IV)—identical with anhydrocycloheximide prepared by known procedures (5).

Catalytic hydrogenation of *epi*-anhydrocycloheximide (III) with a palladium-on-charcoal catalyst gave *epi*-deoxycycloheximide (V) which upon further hydrogenation with a platinum catalyst gave *epi*-dihydrodeoxycycloheximide (VI). Compound VI could also be prepared by the direct hydrogenation of III with a platinum catalyst. When VI was allowed to react with phosphorus tribromide in dioxane solution, the corresponding bromo compound VII was obtained which after reaction with zinc and acetic acid

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<sup>1</sup> In order to distinguish the degradation products which we have prepared in this research from the degradation products which have previously been prepared (5, 7), we have used the prefix *epi* for our new compounds. In each case, the *epi* compounds have been shown to be different from the previously prepared degradation products by comparing the infrared and ultraviolet spectra, melting points and mixture melting points, and by thin-layer chromatography.