fuged, the liquid decanted into another cooled culture tube, and the solid was washed 2–3 times with butane and centrifuged as before. The combined centrifugates were again chlorinated to cause any ester previously trapped in the solid to react and the solid was removed. After being combined, the butane solutions were allowed to evaporate and the residue was distilled using an 18-in. Vigreux column. Evaporation of the butane tended to sweep out some of the more volatile chlorination products. It was found that this loss could be minimized by condensing the butane in a clean tube, again allowing it to evaporate and combining the residues before distillation.

Substances Chlorinated and Products Formed

1. **Methyl thiolbenzoate**, $C_6H_5COSCH_3$, yielded: (a) benzoyl chloride, b.p. 194–195°, 78–79° (15 mm.), $n^{25}D$ 1.5498, d^{25}_4 1.21 (86% yield); (b) methylsulfur trichloride, CH_3SCl_3. *Anal.* Calcd. for CH_3Cl_3S: Cl, 69.31. Found: Cl, 68.52, 70.17.

2. **Ethyl thiolacetate**, $CH_3COSC_2H_5$, yielded: (a) acetyl chloride, CH_3COCl; reacted readily with aniline to form acetanilide, m.p. 112°, mixed m.p. with an authentic sample 112°; (b) ethylsulfur trichloride, $C_2H_5SCl_3$. The white solid was hydrolyzed in cold sodium bicarbonate solution and the solution treated with benzyl chloride to form ethyl benzyl sulfone, $C_2H_5SO_2CH_2C_6H_5$, m.p. 84.5, mixed m.p. with authentic sample 84.5°.

3. **Methyl dithiopropionate**, $C_2H_5C(=S)SCH_3$, yielded: (a) 1,1-dichloropropane-1-sulfenyl chloride, $C_2H_5CCl_2SCl$, yellow liquid, b.p. 69.5° (29 mm.), 65° (27 mm.), $n^{25}D$ 1.510; d^{25}_4 1.361, d^0_4 1.391 (27% yield). *Anal.* Calcd. for $C_3H_5Cl_3S$: Cl, 59.26; MR_D 38.7; mol. wt., 179.5. Found: Cl, 59.64; MR_D 39.4; mol. wt., 175.7. (b) Methylsulfur trichloride, CH_3SCl_3, which was hydrolyzed to sodium methanesulfinate and caused to react with benzyl chloride to form benzyl methyl sulfone, m.p. 126° and unchanged when mixed with an authentic sample.

4. **Methyl dithioacetate**, $CH_3C(=S)SCH_3$, yielded: (a) yellow liquid boiling 46° (28 mm.), presumably methyldichloromethanesulfenyl chloride, CH_3CCl_2SCl, but which decomposed with the evolution of hydrogen chloride too readily to be purified; (b) methylsulfur trichloride which was identified as methyl benzyl sulfone.

5. **Methyl methylxanthate**, $CH_3OCSSCH_3$, yielded: (a) methoxydichloromethanesulfenyl chloride, CH_3OCCl_2SCl, yellow liquid, b.p. 77° (35 mm.), 82° (40 mm.), $n^{25}D$ 1.518, d^{25}_4 1.522, d^0_4 1.555 (70% yield). *Anal.* Calcd. for $C_2H_3OCl_3S$: Cl, 58.62; S, 17.61; MR_D 35.8; mol. wt., 185.1. Found: Cl, 59.06, 58.30; S, 17.4; MR_D 36.2; mol. wt., 181.5; (b) methyl sulfur trichloride, CH_3SCl_3, identified as methyl benzyl sulfone as described above.

6. **Ethyl ethylxanthate**, $C_2H_5OCSSC_2H_5$, yielded: (a) ethoxydichloromethanesulfenyl chloride, $C_2H_5OCCl_2SCl$, yellow liquid, b.p. 88° (33 mm.), $n^{25}D$ 1.507, d^{25}_4 1.422, d^0_4 1.454. *Anal.* Calcd. for $C_3H_5OCl_3S$: Cl, 54.40; MR_D 40.5; mol. wt., 195.5. Found: Cl, 54.55; MR_D 40.9; mol. wt., 195.1. (b) Ethylsulfur trichloride, $C_2H_5SCl_3$. *Anal.* Calcd. for $C_2H_5Cl_3S$: Cl, 63.5. Found: Cl, 61.7, 62.2.

7. **Methyl 1-propylxanthate**, $CH_3CH_2CH_2OCSSCH_3$, yielded: (a) 1-propoxydichloromethanesulfenyl chloride, $CH_3CH_2CH_2OCCl_2SCl$, yellow liquid, b.p. 95.5° (26 mm.), $n^{25}D$ 1.498, d^{25}_4 1.353, d^0_4 1.383. *Anal.* Calcd. for $C_4H_7OCl_3S$: Cl, 50.77; MR_D 45.1; mol. wt., 209.5. Found: Cl, 50.95; MR_D 45.4; mol. wt., 203.1. (b) Methyl sulfur trichloride, CH_3SCl_3, identified by transforming to methyl benzyl sulfone.

8. **Methyl 2-propylxanthate**, $(CH_3)_2CHOCSSCH_3$, yielded: (a) methylsulfur trichloride, CH_3SCl_3, identified by transforming to methyl benzyl sulfone; (b) a yellow liquid boiling 87° (25 mm.) and presumably 2-propoxydichloromethanesulfenyl chloride, $(CH_3)_2CHOCCl_2SCl$, but the product lost hydrogen chloride so readily that purification was impossible.

9. **Bis-[methoxythiocarbonyl] disulfide**, $CH_3OC(=S)SSC(=S)OCH_3$, yielded: (a) methoxydichloromethanesulfenyl chloride, CH_3OCCl_2SCl, yellow liquid, $n^{25}D$ 1.518 (53% yield); (b) a white solid of unknown identity melting with decomposition at 15° and hydrolyzing in water to give a sulfur-like yellow solid. The gases from the decomposition liberated iodine from potassium iodide. The original white solid was analyzed repeatedly but with inconsistent results. Found: Cl, 55.8 to 60.65; S, 20.2 to 25.1.

10. **Bis-[ethoxythiocarbonyl] disulfide**, $C_2H_5OC(=S)SSC(=S)OC_2H_5$, yielded: (a) ethoxydichloromethanesulfenyl chloride, $C_2H_5OCCl_2SCl$, yellow liquid, b.p. 83° (27 mm.), $n^{25}D$ 1.507, d^{25}_4 1.421; (b) a white solid of unknown identity, similar to that obtained from the chlorination of bis-[methoxythiocarbonyl] disulfide.

11. **Methyl methanethiosulfonate**, $CH_3SO_2SCH_3$, yielded: (a) methanesulfonyl chloride, CH_3SO_2Cl, which was allowed to react with aniline to form methanesulfonanilide, $CH_3SO_2NHC_6H_5$, m.p. 97–98° and unchanged when mixed with an authentic sample; (b) methylsulfur trichloride, CH_3SCl_3, which was identified by transformation to methyl benzyl sulfone.

12. **Methyl thionpropionate**, $C_2H_5C(=S)OCH_3$, yielded: a white solid, decomposing at 8° with evolution of a gas which fumed slightly in moist air but which also liberated iodine from potassium iodine–starch paper. *Anal.* Calcd. for $C_2H_5CSOCH_3Cl_2$: Cl, 44.57. Found: Cl, 44.2.

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The Reaction of 2,4-Dinitrobenzenesulfonic Acid with Free Amino Groups of Proteins

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In a previous communication[1] which dealt with the combination of a series of substituted 2,4-dinitrobenzenes with tissue proteins *in vivo*, it was reported that the sodium or potassium salts of 2,4-dinitrobenzene sulfonate formed a stable derivative *in vitro* with bovine gamma globulin. Because the reaction between this compound and protein may readily be carried out under conditions which cause little or no denaturation of many proteins, we have examined this reaction in greater detail.

Dinitrobenzene sulfonate reacts readily with bovine gamma globulin at *p*H 10–11 at room temperature; at *p*H 7, however, under otherwise similar conditions, protein is not derivatized after 24 hours. Since dinitrobenzene sulfonate is appreciably water soluble, the reaction may be carried out in an aqueous system, in which case the derivatized protein is soluble at *p*H 7.0 as well as at higher *p*H values.

The derivatization involves the splitting out of sulfonate and the substitution of dinitrophenyl in free NH_2 groups, yielding the same derivative as in the reaction with 2,4-dinitrofluorobenzene.[2] This conclusion is based upon evidence from three sources.

(1) Bovine gamma globulin was reacted with dinitrobenzene sulfonate and, after purification by extensive dialysis, the protein was hydrolyzed in 6 *N* HCl. After ether extraction, the hydrolysate was examined chromatographically on buffered silica gel columns.[2,3] A single yellow band was obtained with the same R_f as a sample of ε-dinitrophenyllysine prepared by the method of Porter and Sanger[4]; a mixed chromatogram of the latter

(1) H. N. Eisen, L. Orris and S. Belman, *J. Exp. Med.*, **95**, 473 (1952).

(2) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

(3) S. Blackburn, *ibid.*, **45**, 579 (1949).

(4) R. R. Porter and F. Sanger, *ibid.*, **42**, 287 (1948).

compound and the protein hydrolysate yielded only a single band. ε-Dinitrophenyllysine is thus the predominant yellow dinitrophenylamino acid in bovine gamma globulin derivatized with dinitrobenzene sulfonate. Probably free NH_2 groups of terminal amino acids are substituted in addition to the ε-NH_2 of lysine.

(2) The ultraviolet absorption spectrum of the derivatized protein in 10 *N* HCl was corrected for the protein contribution to yield the absorption spectrum of the substituted amino acids. The latter appears not to be significantly different from that of ε-dinitrophenyllysine (Fig. 1); the close approximation of the two spectra in the region of 300 to 340 mμ suggests that little, if any, reaction with tyrosine hydroxyl groups has occurred.[5]

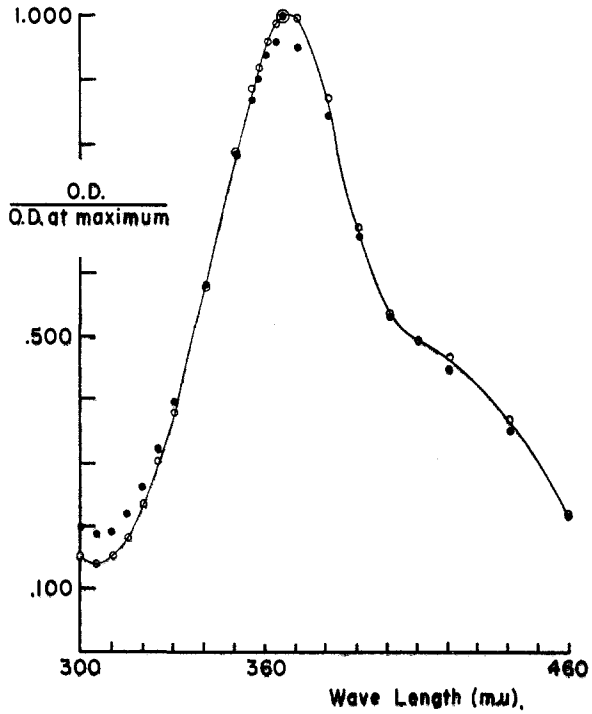

Fig. 1.—Ultraviolet absorption spectrum of ε-dinitrophenyllysine, O, and the dinitrophenylamino acids, ●, of dinitrophenyl bovine gamma globulin; the latter was obtained by correcting the spectrum of the derivatized globulin for the protein contribution. Optical densities (O.D.) are expressed as a fraction of the optical density at λ_{max} (365 mμ). The solvent was 10 *N* HCl.

(3) S^{35}-labeled dinitrobenzene sulfonate[6] (2 mc./m*M*) was diluted with non-radioactive carrier and treated with bovine gamma globulin in an aqueous system, *p*H 10–11. After 24 hours the solution was dialyzed against successive changes of 0.16 *M* NaCl, the dialysates being measured for S^{35} content. When no further S^{35} was dialyzable the dialysis bag contents were analyzed for S^{35} activity and, after dilution in 0.1 *N* NaOH, were examined spectrophotometrically at 290 and 360 mμ in order to estimate the number of dinitrophenyl groups per protein molecule.[1] The results (see Table I) indicate that an average of 22% of dinitrobenzene sulfonate reacted with the protein, while only 1.6% of S^{35} was combined. This amount of S^{35} corresponds to 5 to 10% of the dinitrophenyl groups in the derivatized protein. Inasmuch as the labeled reagent used in these experiments contained an S^{35} impurity to the extent of 10%,[6] the non-dialyzable radioactivity can probably be attributed to this impurity. The impurity has not been identified; despite its presence, it was possible to recrystallize the S^{35}-labeled dinitrobenzene sulfonate six times from water without a change in specific activity.

It is of interest that the efficiency of derivatization of protein by dinitrobenzene sulfonate was increased considerably by shaking the reactants although these were all in solution. For example, preparations A and B of Table I represent aliquots from a single solution of reactants, A having been shaken about 180 times per minute for about 20 hours, and B having been unagitated during the same time.

TABLE I

S^{35}-LABELED DINITROBENZENE SULFONATE REACTION WITH PROTEIN

Initially, labeled reagent was in 86-fold mole excess with respect to bovine gamma globulin.[6] Total S^{35} activity, 30,810 c.p.m. for A, and 53,495 c.p.m. for B.

Preparation	Dinitrophenyl groups per dinitrophenyl-bovine globulin molecule[a]	Fraction of initial dinitrophenyl groups combining with protein (= *x*), %	Fraction of total S^{35} not dialyzable (= *y*), %	Ratio *y*/*x*
A	24	28	2.4	0.086
B	14	16	0.9	.056

[a] Assuming bovine gamma globulin molecular weight to be 160,000.

Under conditions which lead to maximal substitution (*i.e.*, vigorous shaking in the presence of ethanol[2]) 2,4-dinitrofluorobenzene and the corresponding chloro and bromo reagents introduce about 60 dinitrophenyl groups per γ-globulin molecule, and the protein is rendered insoluble.[1] Dinitrobenzene sulfonate, dissolved in water, is about as effective when a large excess of reagent is employed. For example, when used in a 1000-fold mole excess with respect to protein (at *p*H 10 for 20 hours at room temperature, rocking gently about five times per minute) the sulfonate reagent substituted 50 to 55 dinitrophenyl groups per bovine γ-globulin molecule, the protein remaining soluble in water at *p*H values above 6.8. For purposes of end group analysis where denaturation is not a matter of concern, the halogen substituted reagents are probably more satisfactory. Dinitrobenzene sulfonate, however, offers a notable advantage in that it permits the preparation of soluble dinitrophenyl proteins. The latter have been found useful in immuno-chemical work,[7] and may be of interest generally in relation to the preparation of undenatured derivatized proteins.

(5) F. Sanger, *Biochem. J.*, **45**, 563 (1949).

(6) The potassium salt of S^{35}-labeled dinitrobenzene sulfonate was prepared at Technical Associates, Inc., under the direction of Mr. Allen Goldstein; it may now be obtained from Isotopes Specialties Co., Glendale, California. The manufacturer reported that radioautographs of paper chromatograms of this material revealed an impurity, containing S^{35}, to the extent of 10%; the concentration of this impurity was not reduced by numerous recrystallizations.

(7) H. N. Eisen, M. E. Carsten and S. Belman, *Federation Proc.*, **12**, 441 (1953).

In addition to bovine gamma globulin, we have reacted dinitrobenzene sulfonate with egg albumin, beef serum, sheep serum, gelatin and tuberculin with results similar to those given above.

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4-Nitro-2-thenaldehyde

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The nitration of 2-thenaldehyde diacetate has been reported to give 5-nitro-2-thenaldehyde diacetate.[1,2] Since Rinkes[3] found that nitration of methyl 2-thienyl ketone gave a mixture of methyl 5-nitro-2-thienyl ketone and methyl 4-nitro-2-thienyl ketone, it was thought that the nitration of 2-thenaldehyde diacetate should produce 4-nitro-2-thenaldehyde diacetate as well as the 5-nitro derivative. It was found that this was indeed the case. Concentration of the alcoholic filtrates from the recrystallization of the 5-nitro-2-thenaldehyde diacetate, m.p. 72–73°, yielded another nitrothenaldehyde diacetate, m.p. 54–55°. Acid hydrolysis of the latter gave the nitroaldehyde, m.p. 36–37°. Oxidation of the nitroaldehyde to the nitroacid, followed by esterification with methanol, gave a methyl ester, m.p. 99°, corresponding to the melting point of methyl 4-nitro-2-thiophenecarboxylate reported by Rinkes.[4]

Experimental

Since the conditions for the nitration of 2-thenaldehyde diacetate differ in some respects from the procedure used by Patrick and Emerson,[1] these differences are reported here.

To 143 g. of acetic anhydride at −5° was added, over a period of 17 minutes, 43.7 g. of fuming nitric acid (sp. gr. 1.5), the temperature being kept at −5 to 0°. A solution of 55 g. of 2-thenaldehyde diacetate in 102 g. of acetic anhydride was then added slowly, keeping the temperature a −5 to −10°. After addition was complete, the solution was stirred at 0° for three hours and then poured onto 1.0 kg. of ice. The mixture was allowed to stand for one hour and was then filtered. The precipitate was washed with a little cold water followed by cold alcohol and then dried, yielding 58.5 g., 88%, of the isomeric 5-nitro-2-thenaldehyde diacetates, m.p. 55–65°.[5] Three recrystallizations from alcohol gave a 64% yield of 5-nitro-2-thenaldehyde diacetate,[6] m.p. 68–69°.

4-Nitro-2-thenaldehyde Diacetate.—The combined alcoholic filtrates from the first two recrystallizations of the 5-nitro-2-thenaldehyde diacetate were evaporated to dryness. The residue was recrystallized from 18 cc. of alcohol, giving 5.2 g. of solid, m.p. about 50°. The 5.2 g. were recrystallized again from 7 cc. of alcohol, giving 4.0 g. of solid, m.p. 50–55°. A third recrystallization from 10 cc. of alcohol, gave 3.7 g., m.p. 54–55°. Crystallization of a sample from petroleum ether did not further raise the melting point. The ultraviolet absorption maximum in water occurred at 2925 Å., E_M 6,300.

Anal.[7] Calcd. for $C_9H_9NO_6S$: C, 41.70; H, 3.50; N, 5.40; S, 12.37. Found: C, 41.81; H, 3.37; N, 4.97; S, 12.31.

4-Nitro-2-thenaldehyde.—To a solution of 12.5 g. of sulfuric acid in 25 cc. of water was added 6.7 g. of 4-nitro-2-thenaldehyde diacetate. The mixture was refluxed in an atmosphere of nitrogen for 20 minutes, cooled and the resulting precipitate removed by filtration and then washed with cold water. It was recrystallized from a mixture of ether–petroleum ether to give 2.0 g., 50% of 4-nitro-2-thenaldehyde, m.p. 34–37°. Further recrystallization from petroleum ether raised the melting point to 36–37°. The ultraviolet absorption maximum in water occurred at 3025 Å., E_M 7,600.

Anal. Calcd. for $C_5H_3NO_3S$: C, 38.21; H, 1.92; N, 8.92; S, 20.40. Found: C, 37.74; H, 1.94; N, 8.95; S, 20.50.

The semicarbazone melted at 234–235°. A mixed melting point with the 5-nitro-2-thenaldehyde semicarbazone was depressed to 225–230°.

Anal. Calcd. for $C_6H_6N_4O_3S$: C, 33.64; H, 2.82; S, 14.97. Found: C, 33.87; H, 3.07; S, 14.69.

A similar hydrolytic procedure when applied to 5-nitro-2-thenaldehyde diacetate gave a 95% yield of 5-nitro-2-thenaldehyde,[8] m.p. 70–72°.

Methyl 4-Nitro-2-thiophenecarboxylate.—To a suspension of 0.5 g. of 4-nitro-2-thenaldehyde in 3 cc. of 35% sulfuric acid was added dropwise a solution of 0.8 g. of sodium dichromate in 0.5 cc. of water, keeping the temperature below 40°. The mixture was stirred for 3 hours at room temperature and then kept at 0° for 15 hours. The precipitate was removed by filtration, washed with a little cold water, and then dried. It was dissolved in 5 cc. of methanol, 0.1 g. of sulfuric acid added and the solution refluxed for 3 hours. At the end of this time the solution was poured into 15 cc. of ice-water and sufficient sodium bicarbonate solution added to neutralize any excess acid. The solid which formed was removed by filtration and recrystallized twice from petroleum ether. The melting point was 98–99° (99°[4]).

Anal. Calcd. for $C_6H_5NO_4S$: S, 17.13. Found: S, 17.44.

(1) T. Patrick and W. Emerson, THIS JOURNAL, **74**, 1356 (1952).

(2) V. M. Zubarovskii, *Doklady Akad. Nauk S.S.S.R.*, **83**, 85 (1952); *C. A.*, **47**, 2166[a] (1953).

(3) I. Rinkes, *Rec. trav. chim.*, [4] **52**, 538 (1933).

(4) I. Rinkes, *ibid.*, [4] **51**, 1134 (1932).

(5) All melting points were taken on a Fisher–Johns apparatus and are corrected.

(6) The ultraviolet absorption maximum, in water, of an analytically pure sample occurred at 3190 Å., E_M 8,300.

(7) All the analyses were carried out by Mr. Joseph Corrado of these laboratories.

(8) The ultraviolet absorption maximum, in water, of an analytically pure sample occurred at 3150 Å., E_M 11,200.

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The Resolution of *p*-Ethylphenylmethylcarbinol. Infrared Spectra of Enantiomorphs and Racemates

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In connection with another problem we had occasion to resolve *p*-ethylphenylmethylcarbinol. The resolution was carried out by crystallization of the brucine and cinchonidine salts of the acid phthalate of the alcohol[1] and is described in detail in the experimental part. Melting point data of the active and racemic phthalates indicate that the racemic phthalate is a *dl*-compound.

Routine examination of the infrared spectra of the enantiomorphs and racemic phthalates revealed that while the (+)-, (−)- and racemic phthalates had identical spectra in chloroform solution—as was to be expected[2a] the mull spectrum of the racemate (see Fig. 1) showed significant differences

(1) A. W. Ingersoll in R. Adams, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 376.