The **picrate** crystallized from ethanol as shiny platelets, m.p. 136.5–137.5° (after slight sintering).

Anal. Calcd. for $C_{16}H_{17}N_5O_8$: C, 47.17; H, 4.20. Found: C, 46.95; H, 4.23.

The **oxime** was prepared as described for (3-methyl-2-pyridyl)-acetoxime above. It could not be crystallized. The infrared absorption spectrum confirmed the structure of the oily material.

2-Amino-1-(6-dimethylamino-2-pyridyl)propane.—A solution of 5 g. of crude (6-dimethylamino-2-pyridyl)acetoxime in 30 ml. of anhydrous ether was reduced with 8 g. of lithium aluminum hydride as described above. The colorless oily reaction product weighed 3.6 g. (78%) and boiled at 94–96° (1.3 mm.). The dihydrochloride crystallized from methanol–ether as a somewhat hygroscopic colorless material, m.p. 241–242.5° dec.

Anal. Calcd. for $C_{10}H_{19}Cl_2N_3$: C, 47.62; H, 7.59. Found: C, 47.63; H, 7.44.

Reaction of 3,4-Lutidine with Phenyllithium.—A solution of 26 g. of dried redistilled 3,4-lutidine in 100 ml. of anhydrous ether was dropped into a stirred solution of 0.25 *N* phenyllithium,[19] and the mixture was stirred at 25° for 19 hr. After hydrolysis with 100 g. of ice it was extracted with cold 18% hydrochloric acid, and the basic fraction liberated by alkalinization and extraction with ether. The dried extracts were fractionated. Besides 9 g. of unchanged starting material, a yellowish oil (8.1 g.), b.p. 102–123° (1.6–1.7 mm.) was obtained. This was refractionated to give two products, III and IV.

III (presumably 4-benzyl-3-picoline) weighed 2.1 g. The colorless oil had a bitter odor, b.p. 113–115° (2.2 mm.). Its *picrate* crystallized as fine needles from ethanol, m.p. 204–205°.

Anal. Calcd. for $C_{19}H_{16}N_4O_7$: C, 55.33; H, 3.91. Found: C, 55.55; H, 4.19.

IV (presumably 2-phenyl-3,4-lutidine) weighed 2.3 g., b.p. 124–129° (2.2 mm.). The *hydrochloride* crystallized as colorless needles from methanol, m.p. 235–236° dec.

Anal. Calcd. for $C_{13}H_{14}ClN$: C, 71.06; H, 6.42. Found: C, 70.98; H, 6.45.

The *picrate* crystallized from ethanol as yellow leaflets, m.p. 172.5–174.5°.

Anal. Calcd. for $C_{19}H_{16}N_4O_7$: C, 55.33; H, 3.91. Found: C, 55.21; H, 4.10.

Oxidation Experiments.—(a) To a stirred mixture of the oily III (1.5 g.) and 30 ml. of water, 9 g. of powdered potassium permanganate was added at 20° in 0.5 g. portions over a period of 4 hr. The solution was filtered, concentrated to 10 ml., acidified to pH 1 with nitric acid, and the precipitate was filtered and recrystallized from water. It weighed 0.58 g. (68%) and was identified as benzoic acid by its melting and mixture melting points and infrared spectrum. No basic or other product could be isolated from the filtrates *via* a copper salt or after treatment of buffered evaporation residues with diazomethane.

(b) The oily fraction IV was oxidized likewise. The only product to be isolated was benzoic acid, yield, 65.5%.

(c) A solution of 3 g. of III in 400 ml. of 5% sulfuric acid was oxidized, in small portions, with 7 g. of potassium permanganate, first with cooling, then at 25° for 1 hr. It was cleared with 2 g. of sodium bisulfite, acidified with hydrochloric acid, and 0.16 g. of a colorless precipitate (identified as benzoic acid) was filtered.

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The Synthesis of S^{35}-(1,2-Dichlorovinyl)-L-Cysteine[1a]

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In their search for the toxic factor in trichloroethylene-extracted soybean oil meal which induces aplastic anemia[2,3] in cattle, McKinney, *et al.*, synthesized a cysteine derivative[4,5] which when fed in small amounts produces this blood dyscrasia in calves. Based on the method of synthesis and other evidence they identified their product as S-(*trans*-1,2-dichlorovinyl)L-cysteine (DCVC). In this laboratory the extremely high toxicity of DCVC for calves has been amply confirmed,[6] as little as 2 mg./kg. of body weight injected intravenously being sufficient to induce fatal aplastic anemia. Among other species the rat is much more resistant and does not develop a blood dyscrasia[7]; *Escherichia coli* B is highly susceptible.[8]

For studies on the metabolism of DCVC in various species and of the interaction of the whole or fragments of this molecule with components of biological systems, radioactively labeled DCVC of high specific activity was required. Inasmuch as the D-isomer of DCVC is biologically less active than its enantiomorph,[9] synthesis of the radioactively labeled L-isomer is a prerequisite for meaningful biochemical studies.

S^{35} from BaS^{35} was incorporated into DCVC by the sequence of reactions.

$$BaS^{35} + 2H^+ \rightarrow H_2S^{35} + Ba^{++} \quad (1)$$

$$H_2S^{35} + KOH \rightarrow KS^{35}H + H_2O \quad (2)$$

$$C_6H_5CH_2Cl + KS^{35}H \rightarrow \underset{(I)}{C_6H_5CH_2S^{35}H} + KCl \quad (3)$$

$$\underset{(II)}{\text{L-}CH_2ClCHNH_2COOH.HCl} + C_6H_5CH_2S^{35}H \xrightarrow{NaOEt} \underset{(III)}{\text{L-}C_6H_5CH_2S^{35}CH_2CHNH_2COOH} \quad (4)$$

$$(III) \xrightarrow[2.\ CHCl{=}CCl_2]{1.\ Na + NH_3} \underset{(IV)}{\text{L-}CHCl{=}CClS^{35}CH_2CHNH_2COONa} \quad (5)$$

S^{35}-Benzyl-L-cysteine (III) and DCVC, the latter obtained by acidifying aqueous solutions of (IV), were isolated in crystalline form, recrystallized and their identity was compared with that of authentic non-radioactive specimens by melting point, absorption spectra, and chromatography, using the ninhydrin and iodoplatinate reactions and the radioactivity to locate the compounds, and as criteria of purity.

Oral or parenteral administration of S^{35}-DCVC led to rapid excretion of radioactivity in the urine and feces, which was maintained at a low level for a long time. The data shown in Fig. 1 were obtained with a male rat weighing 217 g. which was injected intraperitoneally with 2.56 mg. of DCVC and 6 million counts per minute of total radioactivity. A similar animal to which the same amount of S^{35}-DCVC was given by stomach tube had a 1.5-fold higher excretion of radioactivity in the feces during the first 24 hours. Thereafter it was

(1) (a) Paper No. 4889, Scientific Journal Series, Minnesota Agricultural Experiment Station. Supported in part by the U. S. Atomic Energy Commission (Contract AT (11-1)-364). An abstract of oral report appeared in *Fed. Proc.*, **19**, 7 (1960); (b) postdoctorate trainee USPH Training Grant No. 2G-345; (c) to whom correspondence should be addressed.

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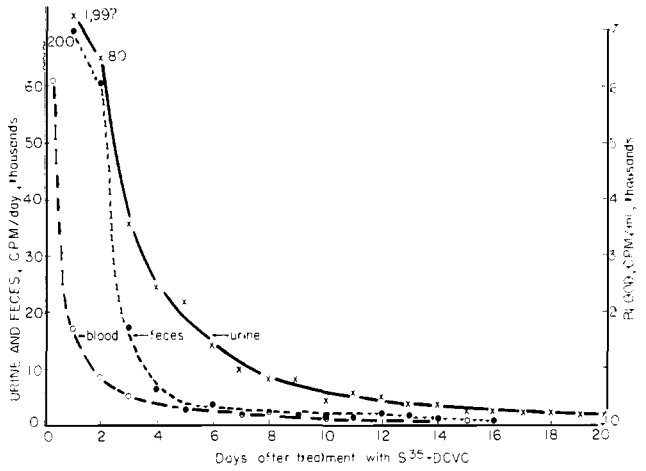

Fig. 1.—Radioactivity (CPM) of blood, urine and feces of rat treated intraperitoneally with S^{35}-DCVC.

essentially the same in both rats. In contrast, the total urinary excretion of S^{35} by the orally treated rat was, during the first 24 and 72-hour intervals, only 40% of that excreted by the parenterally treated animal. The radioactive components in the urine included inorganic sulfate, DCVC and its N-acetyl derivative as reported elsewhere.[10] The fact that in spite of a high rate of clearance of DCVC through the kidneys, excretion of the S^{35} isotope continued for a long period indicates that DCVC or a sulfur compound derived from it combined with components of tissues and thus escaped the initial rapid clearance from the blood stream.

Experimental

S^{35}-Benzyl Mercaptan (I).—The procedure of Tarver and Schmidt[11] was adapted for use of BaS^{35} (30 mc.)[12] and scaled down to obtain preparations of high specific activity. The reactions were carried out under a slow stream of nitrogen in a closed system from which emerging gas passed through a solution of ethanolic 2% $HgCl_2$.

S^{35}-Benzyl-L-cysteine (III).—Methyl 2-amino-3-chloro-L-propionate was prepared according to Fischer and Raske[13] except that diethyl ether was substituted for petroleum ether to wash the product. This ester (800 mg.) was hydrolyzed with 135 ml. of 5.8 *N* HCl and the solution concentrated to dryness *in vacuo*. The residue of 2-amino-3-chloro-L-propionic acid hydrochloride (II) was dissolved in 5 ml. of absolute ethanol and injected with a long needle and syringe through the vented stopper into the flask containing S^{35}-benzyl mercaptan (I).[14] The mixture was stirred by a continuous flow of nitrogen which escaped through the vent into a 2% ethanolic solution of $HgCl_2$. Sodium ethoxide (0.5 g. of Na/10 ml. of absolute ethanol) was added dropwise until the thymolphthalein indicator turned blue and 0.4 ml. more was added. The flask was then heated at 72° for 2 hr. and more sodium ethoxide and/or ethanol added as needed. After cooling, the precipitates of NaCl and KCl were centrifuged, the supernatant removed, and the precipitate washed 3 times with absolute ethanol. The supernatant and washings were evaporated to dryness under a stream of nitrogen while warming the flask to 40°. The vapors were passed through an ethanolic $HgCl_2$ trap. The residue was dissolved in 30 ml. of distilled water and allowed to stand overnight at room temperature. The aqueous solution was extracted 5 times with a total of 40 ml. of ether to remove dibenzyl sulfide and disulfide which were present as solids or oils. The ether extracts were combined and extracted 3 times with water, the first washing being done with water containing a drop of *N* NaOH. The aqueous extracts were combined with the extracted aqueous solution, adjusted to pH 5.5 with HCl (pH paper) and concentrated by drawing air across the surface with slight warming. The concentrated solution was cooled in a refrigerator to obtain crystalline III. It was filtered, washed with cold 50% ethanol, ethanol, ether, and dried *in vacuo* over P_2O_5. The supernatant and washings were concentrated to yield a second crop of crystals. S^{35}-Benzyl-L-cysteine (III) was isolated in crystalline form, m.p. 209–211° dec. (corr.) from four different preparations in yields from 35 to 50%.

Chromatography in 1-butanol:acetic acid:water (12:3:5 v./v.) was done on Whatman No. 1 paper, descending, and detection of the compound through the iodoplatinate, or ninhydrin reactions or through scanning for radioactivity. Quantitative measurement of the radioactivity of 1 cm. segments of the chromatograms revealed that more than 99% of the activity was localized in one spot. The R_f and m.p. were identical with those of a non-radioactive specimen prepared from disodium cysteinate and benzyl chloride in liquid ammonia.[15]

S^{35}-(Dichlorovinyl)-L-cysteine (IV).—For synthesis of this compound by a modification of earlier procedures,[5,6] III was dissolved in about 10 ml. of liquid ammonia in a 100 ml. cylindrical centrifuge tube which was cooled in a bath of trichloroethylene–solid carbon dioxide. It was reduced to dibenzyl and disodium S^{35}-L-cysteinate by the addition, with stirring, of small amounts of sodium until a blue color remained for at least 2 min. Trichloroethylene (0.35 ml.) was added, all at once, and the reaction mixture stirred for 45 min. The cooling bath was removed, and the contents of the tube were evaporated to dryness under slight vacuum. The residue was dissolved in 5 ml. of hot water, and transferred to a 25 ml. erlenmeyer flask. The pH was adjusted to 5.4 with HCl (pH paper) and the volume reduced by heating slightly on a hot plate while drawing air across the surface. An equal volume of absolute ethanol was added and the solution was cooled to −5° to induce crystallization. The product was filtered, washed with 50% ethanol, ethanol, and ether; it was dissolved in water, treated with a small amount of Darco G60, filtered and recrystallized from 50% ethanol. Concentration of the filtrates afforded a second crop of crystals.

The radioactive DCVC was obtained in yields up to 37.4%, based on 2.07 m*M* KOH used in reaction (2) above. The product of each of 4 different preparations had m.p. 160° dec. (corr.) and moved as a single component in chromatography using the procedures referred to above. No radioactive impurities could be detected. The specific activities for 4 preparations ranged from 7.44 to 58.8 × 10^6 DPM/mg. as measured with a Nuclear-Chicago D-47 detector fitted with a micromil window and having a counting efficiency of 30.8%.

(10) For identification of the major S^{35}-containing compounds as inorganic sulfate, DCVC and N-acetyl DCVC, paper chromatography, paper electrophoresis and dilution analysis of the eluted fractions were used. In addition, inorganic sulfate was identified through precipitation as barium sulfate and benzidine sulfate. The 2,4-dinitrophenyl derivative of DCVC-S^{35} from urine was identical with an authentic specimen (m.p. 160°). Authentic specimens of N-acetyl DCVC (m.p. 108–109°) and its *m*-toluidide (m.p. 150–151°) served to identify N-acetyl-DCVC-S^{35} from urine. Details of the methods used are described elsewhere (R. F. Derr, Ph.D. Thesis, University of Minnesota, 1960; R. F. Derr and M. O. Schultze, *Biochem. Pharmacol.*, in press).

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N-(γ-L-Glutamyl)aminobenzoic Acids

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Of a group of N-(phenylalkyl)glutamines which were recently prepared and examined for antimicrobial activity, N-benzyl-L-glutamine was an effective antagonist of the natural amino acid.[2] In addition, other

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