TABLE I
$(RO)_2PO(SR')$

R	R	d^{25}_4	Yield, %	Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	B.p. °C.	B.p. Mm.
Ethyl	Phenyl	1.0941	71.1[a]	48.78	48.34	6.10	6.16	182–186	30
Ethyl	β-Chloroethyl	1.2036	67.9[a]	30.97	30.87	6.02	5.88	147	5
Ethyl	Methyl	1.1168	92.4	32.61	32.79	7.07	7.13	145–150	50
n-Propyl	Ethyl	1.0588	95.2	42.48	42.82	8.41	8.23	141–142	25
n-Butyl	Ethyl	1.0264	88.6	47.24	47.45	9.06	9.08	132	4

[a] Yield from crude disulfide.

obtained. The sulfenyl chlorides react at least as readily as acyl halides, which are known to react rapidly and exothermally with tertiary phosphites at room temperature.[4]

Methanesulfenyl chloride,[5,6] benzenesulfenyl chloride[7,8] and β-chloroethanesulfenyl chloride[9] were treated with triethyl phosphite and ethanesulfenyl chloride[10] was added to tri-*n*-propyl and tri-*n*-butyl phosphites.

A somewhat related group of substances was studied by Foss,[11] who found that aromatic sulfenyl chlorides react with sodium dialkyl thiophosphates, $(RO)_2PO{\cdot}SNa$, forming aryl sulfenyl thiophosphates, $Ar{\cdot}S{\cdot}S{\cdot}PO(OR)_2$.

The reaction of sulfur monochloride with triethyl phosphite, in benzene solution at 5°, was also examined for comparison, and evidently proceeded as

$$S_2Cl_2 + 3(EtO)_3P \longrightarrow 2(EtO)_3PS + (EtO)_3PCl_2 \text{ and}$$
$$(EtO)_3PCl_2 \longrightarrow EtCl + (EtO)_2POCl$$

Three moles of the phosphite ester was required to decolorize one mole of the sulfur chloride.

The compounds were used for biological testing in cancer chemotherapy studies. Attention was directed mainly to the β-chloro thioester, $Cl{\cdot}CH_2{\cdot}CH_2{\cdot}S{\cdot}PO(OEt)_2$ as a possible mustard analog.

Experimental

The sulfenyl chlorides were prepared in toluene or chloroform solutions, by chlorinating the mercaptan or disulfide with sulfuryl chloride at ice or Dry Ice temperatures.[5,6] The solution was then treated with the phosphite in the same solvent, though the reverse order of addition could be used.

Reactions carried out at 0° gave poorer yields than those done at Dry Ice–acetone temperatures and the addition occurred satisfactorily in the range −10 to −60°. The course of the reaction could be followed as in a titration by observing the fading of the orange color of the sulfenyl chloride. The preparation of S-ethyl-O-O-dibutyl thiophosphate is given as a representative example, other syntheses being similar.

A solution of 12.2 ml. (0.1 mole) of diethyl disulfide in 50 ml. of toluene was treated dropwise at −30° with a solution of 8.1 ml. (0.1 mole) of sulfuryl chloride in 15 ml. of toluene. The red-orange solution was left 20 minutes in the bath, and then treated dropwise with 54.1 ml. (0.2 mole) of tri-*n*-butyl phosphite in toluene solution. The red-orange color of the sulfenyl chloride was discharged toward the end of the addition.

The liquid was warmed to room temperature and extracted with an excess of dilute sodium carbonate solution, and then with water. The toluene solution was dried (Na_2SO_4), the solvent removed, and the residue distilled. The product had b.p. 132° at 4 mm., and weighed 45 g. or 88.6%.

The other esters are listed in Table I, together with yields, constants and analyses.

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Some Derivatives of 8-Chloro-6-methylquinoline

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Since it has been demonstrated that 2,6,8-trichloroquinoline,[1] 2,6-dichloro-8-methylquinoline[2] and 6-bromo-2-chloro-8-methylquinoline[2] are readily nitrated to give the corresponding 5-nitroquinolines and that no 8-bromo-2-chloro-6-methyl-5-nitroquinoline[3] was isolated when an attempt was made to nitrate 8-bromo-2-chloro-6-methylquinoline, an investigation was undertaken to determine whether 2,8-dichloro-6-methyl-5-nitroquinoline (VII) could be obtained readily by the nitration of 2,8-dichloro-6-methylquinoline (XVII). Although good yields of VII did result from the nitration of XVII, no 8-chloro-2-hydroxy-6-methyl-5-nitroquinoline (XII) was obtained when 8-chloro-2-hydroxy-6-methylquinoline (XVIII) was nitrated. Inasmuch as the failure to produce 5-nitroquinolines from the nitration of 8-bromo-2-hydroxy-6-methylquinoline[3] and 6-bromo-2-hydroxy-8-methylquinoline[2] has been reported previously, the failure to produce XII by the nitration of XVIII is not particularly surprising.

Satisfactory methods for preparing several amino, acetamido and benzamido substituted 8-chloro-6-methylquinolines, and some arsonic acids derived from 8-chloro-6-methylquinoline were accomplished and are described.

Experimental

8-Chloro-6-methyl-5-nitroquinoline (I) A.—8-Chloro-6-methylquinoline (50 g.) (obtained in 62 to 66% yields from 4-amino-3-chlorotoluene under conditions as reported by Richter and Smith[4] for effecting Skraup ring closures) was dissolved in sulfuric acid (62.5 ml., sp. gr. 1.84) and the solution resulting added dropwise with stirring at 0° or less to a solution of nitric acid (45 ml., sp. gr. 1.42) in sulfuric acid (62.5 ml., sp. gr. 1.84). After removing the reaction system from the cooling-bath and allowing it to remain suspended in the atmosphere of the laboratory for ten hours,

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the temperature of the reaction system was slowly increased to 70° and maintained for five minutes before discontinuing the application of heat. When the temperature of the reaction system had spontaneously decreased to 40°, it was poured, with stirring, into a cracked ice–water mixture (2000 ml.). The solid product that was obtained after making the system alkaline by the addition of a water solution of sodium hydroxide and then filtering, was washed with water and dissolved in boiling 95% ethanol. The application of a decolorizing carbon followed by Hiflo Super-cel treatment preceded crystallization as the system cooled; 55 g. (87%) yield of white solid, m.p. 117–118°.

B.—4-Acetamido-3-chloro-6-nitrotoluene (8 g.), dry glycerol (10.9 ml.), arsenic pentoxide (8.3 g.) and sulfuric acid (7.3 ml., sp. gr. 1.84) were mixed well and heated together under reflux by means of an oil-bath maintained at 135–140° for seven hours. The cooled mass was poured with stirring into cracked ice–water mixture and treated with Hiflo Super-cel prior to filtering. The addition of sodium hydroxide to the filtrate caused the precipitation of crude I which was separated by filtration and washed with water. Purification was effected by dissolving in boiling 95% ethanol followed by treatment with decolorizing carbon and Hiflo Super-cel prior to filtering and permitting crystallization to occur; 2 g. (26.7%) yield, m.p. 117–118°.

A mixture of I(A) and I(B) also melted at 117–118°.

Anal. Calcd. for $C_{10}H_7ClN_2O_2$: N, 12.59; Cl, 15.93. Found: N, 12.47; Cl, 15.81.

8-Chloro-1,6-dimethyl-5-nitro-2-quinolone (VI).—I (47 g.) was converted into the corresponding methylquinolinium iodide and oxidized with 30% hydrogen peroxide under conditions similar to those previously reported by de Arce, Greene and Capps[3] for the conversion of 8-bromo-6-methyl-5-nitroquinoline into 8-bromo-1,6-dimethyl-5-nitro-2-quinolone. Small orange crystals (29.5 g., 55.5% yield) separated from a hot ethanolic solution upon cooling; m.p. 129–131°.

Anal. Calcd. for $C_{11}H_9ClN_2O_3$: N, 11.09; Cl, 14.03. Found: N, 10.95; Cl, 11.10.

2,8-Dichloro-6-methyl-5-nitroquinoline (VII). A.—XVII (42.5 g.) was nitrated under conditions similar to those described for the nitration of 8-chloro-6-methylquinoline. Purification was accomplished by crystallization from hot acetone; 47 g. (90.5%) yield, m.p. 193–194°.

B.—VI (29.5 g.) was treated with phosphorus oxychloride–phosphorus pentachloride mixture under essentially the same conditions as those described by de Arce, Greene and Capps[3] for converting 8-bromo-1,6-dimethyl-5-nitro-2-quinolone into 8-bromo-2-chloro-6-methyl-5-nitroquinoline; 6 g. (19%) yield, m.p. 193–194°.

A mixture of VII(A) and VII(B) also melted at 193–194°.

Anal. Calcd. for $C_{10}H_6Cl_2N_2O_2$: N, 10.90; Cl, 27.59. Found: N, 10.83; Cl, 27.54.

2,8-Dichloro-6-methylquinoline (XVII).—8-Chloro-6-methylquinoline (85 g.) was treated with dimethyl sulfate and oxidized in basic solution with potassium ferricyanide under conditions similar to those reported by de Arce, Greene and Capps[3] for the conversion of 8-bromo-6-methylquinoline into 8-bromo-1,6-dimethyl-2-quinolone. Attempts to purify the quinolone by recrystallization from organic solvents were not successful; 93.7 g. (94%) yield, melting range 47–51°.

Crude 8-chloro-1,6-dimethyl-2-quinolone (9317 g.) was treated with phosphorus oxychloride–phosphorus pentachloride mixture under essentially the same conditions as those described by de Arce, Greene and Capps[3] for converting 8-bromo-1,6-dimethyl-2-quinolone into 8-bromo-2-chloro-6-methylquinoline except that the heating was done at 170–175° for 4.5 hours; 60 g. (62.5%) yield, m.p. 115–116°.

Anal. Calcd. for $C_{10}H_7Cl_2N$: N, 6.60; Cl, 33.44. Found: N, 6.65; Cl, 33.32.

8-Chloro-2-hydroxy-6-methylquinoline (XVIII).—XVII (10 g.) was mixed with a solution of sulfuric acid (40 ml., sp. gr. 1.84) in water (25 ml.) and the system refluxed for four hours. The resulting solution was poured upon cooling into a cracked ice–water mixture (100 ml.) accompanied by stirring. The crude XVIII was separated by filtration, washed with water, and purified by crystallizing from hot acetone subsequent to applying a decolorizing carbon–Hiflo Super-cel treatment; 8 g. (87%) of white crystals, m.p. 187–188°.

Anal. Calcd. for $C_{10}H_8ClNO$: N, 7.24; Cl, 18.32. Found: N, 7.17; Cl, 18.26.

8-Chloro-2-hydroxy-6-methyl-5-nitroquinoline (XII).—VII (20 g.) was mixed with a solution of sulfuric acid (160 ml., sp. gr. 1.84) in water (160 ml.) and the system was refluxed for 20 minutes. The resulting solution was poured, upon cooling, into cracked ice–water mixture (1400 ml.) accompanied by stirring. The crude XII was separated by filtration, washed with water and purified by crystallizing from 95% ethanol subsequent to applying a decolorizing carbon–Hiflo Super-cel treatment; 18.9 g. (97.5%); m.p. 245–246°.

Anal. Calcd. for $C_{10}H_7ClN_2O_4$: N, 11.74; Cl, 14.86. Found: N, 11.45; Cl, 14.88.

5-Amino-8-chloro-6-methylquinoline (II).—I (10 g.) was reduced catalytically in acetone at approximately 50° using Raney nickel catalyst, hydrogen at 40 p.s.i. pressure, and shaking for one hour. After removing the catalyst by filtration, dry HCl was passed into the filtrate causing the precipitation of a crimson hydrochloride that was subsequently separated by filtration. The amine hydrochloride was then suspended in water and treated with ammonia water to liberate the amine. Separation of the amine by filtration followed by washing and crystallization from 95% ethanol gave fine yellow crystals; 4.5 g. (52%) yield, m.p. 82–84°.

Anal. Calcd. for $C_{10}H_9ClN_2$: N, 14.55; Cl, 18.41. Found: N, 14.56; Cl, 18.37.

5-Acetamido-8-chloro-6-methylquinoline (III).—II (1.5 g.) was refluxed for 30 minutes with glacial acetic acid (20 ml.) and acetic anhydride (6 ml.) prior to pouring, with stirring, into a cracked ice–water mixture (150 ml.). Several hours later the solid that had formed was removed by filtration, washed with water and purified by crystallization from 95% ethanol subsequent to a decolorizing carbon–Hiflo Super-cel treatment; 98.5% yield of fine white crystals, m.p. 227–228°.

Anal. Calcd. for $C_{12}H_{11}ClN_2O$: N, 11.94; Cl, 15.11. Found: N, 11.72; Cl, 14.88.

5-Benzamido-8-chloro-6-methylquinoline (IV).—II (1 g.) benzoyl chloride (2.5 m.) and 10% aqueous NaOH solution (12.5 ml.) were shaken until the oil that first formed solidified. The solid was removed by filtration, washed with water, and dissolved in boiling 95% ethanol. Small white crystals formed subsequent to a decolorizing carbon–Hiflo Super-cel treatment; 0.8 g. (52%) yield, m.p. 103–105°.

Anal. Calcd. for $C_{17}H_{13}ClN_2O$: N, 9.44; Cl, 11.95. Found: N, 9.27; Cl, 12.18.

8-Chloro-6-methyl-5-quinolinearsonic Acid (V).—II (10 g.) was diazotized and converted into V according to the procedure reported by Capps and Hamilton[5] for changing certain 2-chloroaminoquinolines into 2-chloroquinolinearsonic acids; 1.5 g. (8.9%) yield, m.p. greater than 325°.

Anal. Calcd. for $C_{10}H_9ClAsO_3$: N, 4.65; As, 24.84. Found: N, 4.75; As, 24.75.

5-Amino-2,8-dichloro-6-methylquinoline (VIII).—VII (18.1 g.) was reduced in acetone under conditions similar to those used during the reduction of I; 14.7 g. (92.5%) yield of hydrochloride. Solid amine was liberated from its hydrochloride by treatment with ammonia–water solution. Recrystallization from ethanol–water solution (60:40 by volume) gave yellow VIII, m.p. 178–180°.

Anal. Calcd. for $C_{10}H_8Cl_2N_2$: N, 12.34; Cl, 31.23. Found: N, 12.29; Cl, 31.27.

5-Acetamido-2,8-dichloro-6-methylquinoline (IX).—Conditions similar to those used for converting II into III were used to convert VIII (1.8 g.) into IX, m.p. 284–285°.

Anal. Calcd. for $C_{12}H_{10}Cl_2N_2O$: N, 10.41; Cl, 26.37. Found: N, 10.35; Cl, 26.44.

5-Benzamido-2,8-dichloro-6-methylquinoline (X).—VIII (1 g.) was converted into X by the method used to convert II into IV, m.p. 213–214°.

Anal. Calcd. for $C_{17}H_{12}Cl_2N_2O$: N, 8.46; Cl, 21.41. Found: N, 8.45; Cl, 21.36.

2,8-Dichloro-6-methyl-5-quinolinearsonic Acid (XI).—The conditions used to convert II into V were used to change

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VIII (13.5 g.) into XI; 5.7 g. (30%) yield, m.p. 307–308° dec.

Anal. Calcd. for $C_{10}H_8Cl_2NAsO_3$: N, 4.17; As, 22.29. Found: N, 4.15; As, 21.97.

5-Amino-8-chloro-2-hydroxy-6-methylquinoline (XIII).—XII (19.7 g.) was dissolved in absolute ethanol and reduced catalytically under conditions similar to those used for reducing I and VII. Crude XIII was liberated from its hydrochloride with ammonia–water prior to purifying by crystallization from hot ethanol–water solution (50:50 by volume); 10 g. (58.5%) of yellow crystals, m.p. 195–197°.

Anal. Calcd. for $C_{10}H_9ClN_2O$: N, 13.43; Cl, 17.00. Found: N, 13.26; Cl, 16.99.

5-Acetamido-8-chloro-2-hydroxy-6-methylquinoline (XV).—XIII (1.5 g.) was changed into XV under conditions similar to those used for converting II and VIII into III and IX, respectively. The crude XV was purified by crystallization from hot ethanol–water solution; 1.2 g. (67%) yield, m.p. 305–307°.

Anal. Calcd. for $C_{12}H_{11}ClN_2O_2$: N, 11.18; Cl, 14.14. Found: N, 11.15; Cl, 14.07.

5-Benzamido-8-chloro-2-hydroxy-6-methylquinoline (XIV).—The method that de Arce, Greene and Capps[3] used to convert 5-amino-8-bromo-2-hydroxy-6-methylquinoline into its 5-benzamido derivative was resorted to for changing XIII (1.5 g.) into XIV; 2 g. (92%) yield, m.p. 260–262°.

Anal. Calcd. for $C_{17}H_{13}ClN_2O_2$: N, 8.96; Cl, 11.34. Found: N, 8.79; Cl, 11.32.

8-Chloro-2-hydroxy-6-methyl-5-quinolinearsonic Acid (XVI).—XIII (4.5 g.) was diazotized and converted into XVI under conditions similar to those used for converting II into V and VIII into XI; 1.9 g. (27.8%) yield, m.p. 310–312° dec.

Anal. Calcd. for $C_{10}H_9ClNAsO_4$: N, 4.41; As, 23.59. Found: N, 4.21; As, 23.77.

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Separation of the Glycyl from the Phenylalanyl Chain of Oxidized Insulin by Countercurrent Distribution[1]

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The initial step in the determination of the amino acid sequence of insulin by Sanger and his colleagues[2–4] was a cleavage of the molecule with performic acid which produced two peptide chains, one with phenylalanine as the N-terminal amino acid, the other with glycine in the N-terminal position. The amino acid composition of these chains is such that a separation could be achieved by methods of precipitation utilizing changes in *p*H, or salt, or ethanol concentrations.[2] However, the yields of the two chains were relatively low with a combined recovery of 40–50% of theory. The experiments described here are an application of the technique of countercurrent distribution to the quantitative separation of the two chains to determine the maximum possible yield of the chains which can be obtained by the performic acid cleavage. A complete separation of the glycyl chain from the phenylalanyl chain was achieved by only a few transfers with the solvent system, 2-butanol/aqueous 0.077 *M* *p*-toluenesulfonic acid. Under the best conditions a combined yield of the two chains of approximately 95% has been obtained. The remaining material was mainly a fraction insoluble in the solvent system. The phenylalanyl chain was not separated from any traces of unoxidized insulin which might have been present.

Experimental

Insulin Preparations.—The insulin was beef insulin (Boots Pure Drug Co., Ltd., Nottingham, England). One experiment with essentially the same results was carried out with crystalline beef insulin (Eli Lilly Co.).

Oxidation with Performic Acid.—Oxidations were carried out at −5 to −10°, 25 and 50°. Conditions including those of Sanger,[2] the milder ones of Sanger and Thompson[4] and those with preformed performic acid[5] were used. Initially the reaction mixtures, following oxidation, were diluted with several volumes of water and lyophilized. In later experiments the following procedure led to much less insoluble material and more reproducible results. After oxidation, the reaction mixture was diluted with 10 volumes of water, cooled to 0° and concentrated to a thin sirup with a rotary evaporator[6,7] with 5 ml. ethanol added to prevent foaming. The solvent system was then added immediately. Concentration was completed within 30 minutes after oxidation.

Determination of Insoluble Material.—The insoluble material, which collected at the interphase when oxidized material was placed in the solvent system, was removed before distribution with a dropping pipet and placed in a small centrifuge tube. Then most of the remaining solvent was removed by pipet and dry 2-butanol was added to take up the residual aqueous phase. The insoluble material was centrifuged, washed three times with ether after removal of the butanol, dried and weighed.

Countercurrent Distribution.—Distributions were carried out at room temperature in glass apparatus with 10 or 40 ml. in each phase.[8] From 15 to 50 mg. of material was used in each distribution. The solvent system was prepared by equilibrating equal volumes of 2-butanol and 0.077 *M* *p*-toluenesulfonic acid (Eastman Kodak Co.). Determination of the distribution curves was as previously described[9] with a quantitative ninhydrin reaction[10] after hydrolysis of the peptides. Results are expressed in mg. of peptide determined by comparison with a standard curve of hydrolyzed insulin.

Recovery of Glycyl Chain for Chromatography.—The contents of the tubes containing the slow moving component were combined and taken nearly to dryness at low temperature in the rotary evaporator. *p*-Toluenesulfonic acid and traces of water were removed by repeated extraction with ether. The peptide remained as a dry film on the sides of the flask and was recovered by solution in water. After hydrolysis, two-dimensional paper chromatograms were run on this material and on the insoluble fraction by the ascending method[11] with *n*-butanol–acetic acid[12] and phenol–water[13] solvents.

Results and Discussion

The distribution curve of unoxidized insulin after 49 transfers showed a single peak agreeing

(1) Supported by a research grant, No. C-2290 from National Cancer Institute, U. S. Public Health Service, National Institutes of Health.

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