reported difficulties in obtaining a product with mp 118–120°. It is therefore of interest that, during chlorination, the resulting precipitate of III consisted initially of needles, followed by tiny platelets; prior to extensive washing with Et_2O, the precipitate melted in two steps under a microscope hot stage, mp 94 (platelets) and 102° (needles). These are obviously the two anomers of III and the melting point of the final product will clearly depend on the ratio of the two anomers which, in turn, determines the ratio of the two anomeric nucleosides in the condensation reaction.[19] Because of the lability of III, the freshly prepared mixture of anomers was used as such immediately in the subsequent condensation step.

1-(3,5-Di-O-*p*-chlorobenzoyl-2-deoxy-α,β-D-ribofuranosyl)-5-ethyluracil (IV).—II (1.49 g, 4.4 mmoles) was suspended in about 130 ml of anhydrous PhMe, vigorously stirred, and dried azeotropically by removal of about one-third of the solvent; 3.8 g of III (8.8 mmoles), previously dried, was added rapidly, and the mixture was heated 3 min, then cooled and filtered through glass wool. The precipitate was dissolved ($CHCl_3$), the solution was washed (30% KI, H_2O), and the orgnic phase was dried (Na_2SO_4). The salt was filtered off, the $CHCl_3$ solution was brought to dryness, and the residue was dissolved in hot anhydrous EtOH. Crystallization occurred on cooling to give 1.1 g (47%) of IV, melting at 154–178° and exhibiting under a microscope hot stage two types of crystals.

Attempted Separation of the Anomers of V by Ion-Exchange Chromatography.—A Dowex 1-X2 (200–400 mesh) (OH^-) column, 23 × 2 cm, was washed with 500 ml of 30% aqueous MeOH, and 15 mg of V in the same solvent deposited on the column. The latter was then washed with 250 ml of 60% aqueous MeOH and 250 ml of 90% aqueous MeOH. The nucleoside was then eluted with 0.05 *M* NH_4HCO_3 at a flow rate of about 1.5 ml/min and fractions of 13 ml were collected. The nucleoside appeared in two fairly-well-defined peaks: fraction 13 (4.9 mg, mp 172–174.5°) and fraction 16 (5.2 mg, mp 143–147°). Fraction 16, but not fraction 13, supported bacterial and phage multiplication and is therefore the β anomer of V.

Separation of anomers of IV was achieved on GF_{254} silical gel, deposited as 1.0-mm layers on 20 × 15 cm glass plates, with the solvent system $CHCl_3$–Et_2O (8:2, v/v). It was necessary to run each plate three or four times to obtain adequate separation, the final R_f values for the α and β anomers being about 0.85 and 0.95. The gel containing each of the spots was deposited on a sintered-glass filter and eluted with $CHCl_3$. The eluates were brought to dryness and the residues crystallized from EtOH to give the pure α and β anomers of IV with mp 186–187.5° and 196–197°, respectively.

2′-Deoxy-α(β)-D-ribofuranosyl-5-ethyluracil (V).—The α and β anomers of IV were debenzoylated according to Prystas and Šorm[20] and each was recrystallized (EtOH) to give the α and β anomers of V with mp 177–179° and 152–153°, respectively. The spectral data of the α anomer was as follows: $\lambda_{max}^{pH_2}$ 268 mμ (ϵ_{max} 9.78 × 10^3), $\lambda_{min}^{pH_2}$ 235 mμ (ϵ_{min} 2.42 × 10^3), $\lambda_{max}^{pH_{12}}$ 268 mμ (ϵ_{max} 7.40 × 10^3), $\lambda_{min}^{pH_{12}}$ 245 mμ (ϵ_{min} 4.27 × 10^3); pK_a = 9.86. The spectral data of the β anomer was as follows: $\lambda_{max}^{pH_2}$ 267.5 mμ (ϵ_{max} 9.61 × 10^3), $\lambda_{min}^{pH_2}$ 235 mμ (ϵ_{min} 2.35 × 10^3), $\lambda_{max}^{pH_{11}}$ 267.5 mμ (ϵ_{max} 7.28 × 10^3), $\lambda_{min}^{pH_{12}}$ 245 mμ (ϵ_{min} 4.62 × 10^3); pK_a = 9.98.

Nmr spectra were determined in D_2O for each of the anomers at 30° with a Varian HA-100 spectrometer and Me_4Si as external standard. Except for the added signals due to the presence of a 5-Et in place of a 5-Me substituent, the spectra were similar to those of the corresponding anomers of thymidine;[10] for α-5-ethyldeoxyuridine, 6.51 ppm (H_1, quartet), $J_{H_1'-H_2'}$ = 8.0 cps, $J_{H_1'-H_2''}$ = 3.6 cps; for the β anomer, 6.58 ppm (H_1, triplet) $J_{H_1'-H_2'}$ = $J_{H_1'-H_2''}$ = 7.0 cps.

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(19) B. R. Baker, J. P. Joseph, E. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954).

(20) M. Prystas and F. Šorm, *Collection Czech. Chem. Commun.*, **31**, 1035 1(966).

4,4′,6,6′-Tetrabromo-2,2′-biphenyldiol Mono(dihydrogen phosphate). A New Agent for Combating Distomatosis[1]

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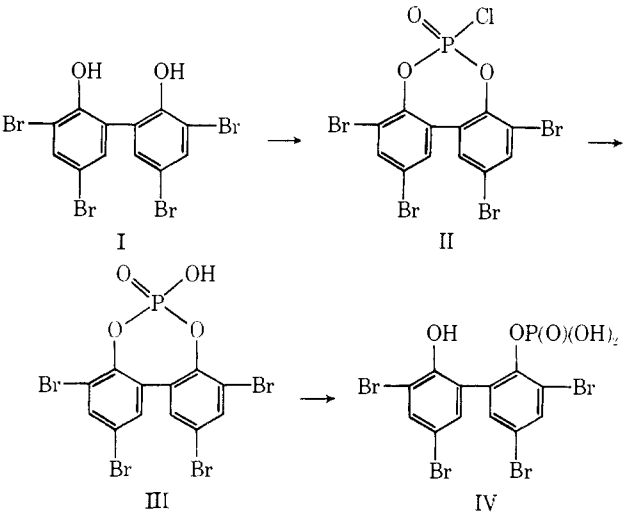
In the course of investigations on agents for combating distomatosis, a disease of sheep and cattle caused by the liver fluke, *Fasciola hepatica*, the title compound (IV) was synthesized according to Scheme I.

SCHEME I



The starting material I was obtained by bromination of 2,2′-biphenyldiol.[2] Treatment with $POCl_3$ in toluene provided the cyclic phosphorochloridate (II). Hydrolysis of II was carried out best by dissolving the crystalline compound in toluene, adding this solution to an aqueous EtOH solution of NaOH, and refluxing the mixture. In this way the poor solubility of the sodium salt of the intermediate III did not interfere. Protracted boiling of the alkaline solution results in complete loss of the acid group. The few per cent of I formed along with the open phosphate IV is easily removed by the purification process described in the Experimental Section.

Pharmacology.—The title compound has been found to be a potent agent in controlling distomatosis. Therapeutic doses of 16 and 12 mg/kg, respectively, in sheep and cattle would require doses of 20 and 16 mg/kg of the standard drug 2,2′-methylenebis(3,4,6-trichlorophenol) to obtain comparable results.[3] The acute toxicity in mice (LD_{50} > 150 mg/kg) was lower than that of the standard. A dose of 36 mg/kg may be safely administered to cattle. Laboratory tests with mice and rats[4] and field trials with some thousands of cattle[5] indicate also activity against immature liver

(1) S. van der Meer, W. Kruyt, and H. Pouwels, Dutch Patent Appl. 65.05635 (1966); corresponding foreign applications are pending.

(2) O. Diels and A. Bibergeil, *Ber.*, **35**, 306 (1902).

(3) W. Kruyt and E. J. van der Steen, *Tijdschr. Diergeneesk.*, **94**, 308 (1969).

(4) W. Kruyt and E. J. van der Steen, to be published.

(5) J. S. Reinders, *Tijdschr. Diergeneesk.*, **94**, 324 (1969).

flukes. After the administration of therapeutic doses, IV could not be detected in the milk.[6]

Experimental Section

4,4′,6,6′-Tetrabromo-2,2′-biphenyldiol Mono(dihydrogen phosphate) (IV).—A mixture of 502 g (1 mole) of the biphenyldiol (I), 1.5 l. of PhMe, 2 ml of pyridine, and 110 ml (1.2 moles) of $POCl_3$ was refluxed for 6 hr. The solution was evaporated *in vacuo* to dryness to remove excess $POCl_3$, preferably repeating the distillation with an additional amount of PhMe to prevent formation in the next step of difficultly hydrolyzable pyrophosphates which would largely prevent the crystallization of the end product IV and diminish the yield. The very hard crystalline cyclic phosphorochloridate (II) was dissolved in 1.5 l. of warm PhMe and this solution was added cautiously with stirring to a solution of 232 g (5.8 moles) of NaOH in 860 ml of H_2O and 300 ml of EtOH. The mixture was boiled for 2 hr and cooled. The aqueous layer was acidified with 0.5 l. of concentrated HCl and extracted (EtOAc). The extract was evaporated *in vacuo* at low temperature to a total weight of 680 g. The syrupy residue was dissolved as rapidly as possible by shaking with C_6H_6 (1.5 l.). Crystallization started in a few minutes. After keeping the mixture at room temperature for at least one night 436 g of crystalline phosphate (IV) was collected. The mother liquor was evaporated to dryness at low temperature, and the residue was dissolved in a small amount of EtOAc and extracted with a solution of 21 g of $NaHCO_3$. From this slightly alkaline solution an additional 64 g of IV could be obtained in an analogous way; yield 500 g (86%). Recrystallization of IV in the usual way is not possible as heating in a solvent is accompanied with gradual cyclization to the more difficultly soluble III.

Compound IV has no melting point; cyclization to III and gradual decomposition occurs on heating up to above 350°. Thorough drying is accompanied by partial dehydration. For analytical purposes the more stable cyclic phosphate (III) was prepared by refluxing for some hours a concentrated solution of IV in EtOAc and isolating the precipitated III.

Anal. Calcd for $C_{12}H_5Br_4O_4P$: C, 25.54; H, 0.89; Br, 56.72; P, 5.50. Found: C, 25.37; H, 0.91; Br, 56.90; P, 5.48.

By potentiometric titration one OH group could be detected in III, whereas IV revealed three OH. Additional support for the given structure of IV is obtained from its ready solubility both in EtOAc and in cold aqueous $NaHCO_3$; III is only very sparingly soluble in these media. The starting material I is readily soluble in EtOAc, not in aqueous $NaHCO_3$.

(6) H. B. de Boer and J. F. Kleinepier, *Neth. Milk Dairy J.*, in press

Nitrofuryl Heterocycles. VIII.[1] 2-(5-Nitro-2-furyl)cinchoninic Acid Derivatives

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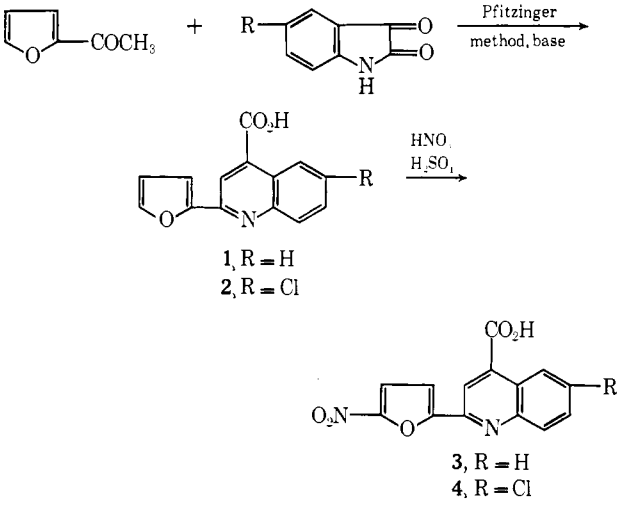
A continuing search for new nitrofuryl heterocycles possessing antimicrobial activity prompted an investigation of 2-(5-nitro-2-furyl)cinchoninic acid derivatives. 2-(2-Furyl)cinchoninic acid (**1**) reportedly has been prepared by the Doebner quinoline synthesis from furfural, pyruvic acid, and aniline.[2] Attempts to duplicate that procedure in this laboratory have failed. However, a 74% yield of **1** was obtained by the Pfitzinger quinoline synthesis from 2-acetylfuran and isatin. Mixed acid nitration of **1** and **2** gave the respective 5-nitro-2-furyl derivatives **3** and **4**. The position of nitration was confirmed by nmr analyses. Acid **3** was characterized further by the formation of its ethyl ester **5** and amide **6**.



The compounds in Table I were screened for antibacterial activity by methods reported previously.[3] Compounds **3**–**6** showed some activity *in vitro* against both gram-positive and gram-negative organisms. Amide **6** was inactive orally in mice against a *Staphylococcus aureus* infection but gave an ED_{50} value of 5 mg/kg when administered intraperitoneally. Acid **4** effectively controlled a *Salmonella gallinarum* infection in chickens at a drug level of 0.011% by weight in feed.

Experimental Section

All melting points were determined on a hot stage (Fisher-Johns) melting point apparatus and are uncorrected. The nmr spectra were determined on a Varian Model A-60 spectrometer in DMSO-d_6 using Me_4Si as an internal standard. The ir spectra were determined as Nujol mulls on a Perkin-Elmer Model 137 spectrophotometer.

2-(2-Furyl)cinchoninic Acid (1).—A solution of 454 g (11.3 moles) of NaOH pellets and 355 g (2.42 moles) of isatin in 3 l. of H_2O was heated for 0.5 hr at 80–90°. With vigorous stirring, 266 g (2.42 moles) of melted 2-acetylfuran was added cautiously in small portions during 0.5 hr. The reaction was very exothermic. Refluxing was continued for 2 hr following the addition after which the mixture was chilled to 0° and filtered through sintered glass. The residual Na salt was dissolved in 2.5 l. of H_2O and the resulting solution was acidified with AcOH. The crude **1** separated as yellow needles decomposing at 228–230°, yield 450 g (77.6%). A decomposition point of 227° has been reported.[2]

Compound **2** was prepared similarly in 45% yield from 2-acetylfuran and 5-chloroisatin; mp 285–287° (AcOH). *Anal.* ($C_{14}H_8ClNO_3$) C, H, Cl.

2-(5-Nitro-2-furyl)cinchoninic Acid (3).—Powdered **1** (50.0 g, 0.21 mole) was added in small portions with stirring to 300 ml of concentrated H_2SO_4 at 0°. When solution was complete, a cold solution of 25 ml of concentrated HNO_3 in 25 ml of concentrated H_2SO_4 was added dropwise during 0.5 hr. Stirring was continued in the cold for 1 hr following the addition. The mixture was then poured cautiously with vigorous stirring into 4 l. of ice–H_2O. The crude **3** was filtered, washed (H_2O), and recrystallized (AcOH, charcoal). The product separated as yellow microneedles decomposing at 281.5–282.5°, yield 32.2 g (54%). *Anal.* ($C_{14}H_8N_2O_5$) C, H, N.

Compound **4** was prepared similarly from **2** in 62% yield; mp 293–295° dec (AcOH). *Anal.* ($C_{14}H_7ClN_2O_5$) C, H, N.

Ethyl 2-(5-Nitro-2-furyl)cinchoninate (5).—Anhydrous HCl was bubbled through a solution of 30 g (0.11 mole) of **3** in 1 l. of EtOH. When solution was complete, the HCl inlet tube was replaced by a stopper and the solution was refluxed for 4 hr.

(1) For the previous paper in this series see H. A. Burch, *J. Med. Chem.*, **11**, 79 (1968).

(2) R. Civsa and F. Bellino, *Gazz. Chim. Ital.*, **66**, 452 (1936).

(3) F. F. Ebetino, W. F. Carey, and B. F. Stevenson, *J. Med. Chem.*, **6**, 633 (1963).