Notes

Antivirals. 1. 2-(α-Hydroxybenzyl)imidazo[4,5-c]pyridine

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The recognition of the antiviral activity of DL-2-(α-hydroxybenzyl)benzimidazole (HBB) in the late 1950's[1] and the completion of the first exploratory studies were promptly followed by investigations of structure–activity relationships. In particular, Tamm and Eggers[2] and O'Sullivan and coworkers[3,4] have explored this area in detail.

Heterocyclic analogs of HBB have, however, been studied little to date.[5,6] Our interest centered therefore on the question of whether the benzene ring of the benzimidazole part is necessary as such for antiviral activity or whether simply the presence of an aromatic electron distribution, as, for example, in a pyridine ring, is sufficient.

Chatterjee, *et al.*,[7] guided perhaps by similar considerations, reacted 3,4-diaminopyridine with mandelic acid and assigned to their product the 2-(α-hydroxybenzyl)imidazo[4,5-c]pyridine structure **8** but found no antiviral activity. This finding surprised us, as we knew from CNDO calculations for 2-(α-hydroxybenzyl)imidazo[4,5-c]pyridine (**8**) that the electron distribution does not differ fundamentally from that of HBB, although the dipole moment is greater.†

These findings induced us to repeat the synthesis as described by Chatterjee, *et al.*,[7] whereby we obtained a compound whose spectroscopic properties were not in accord with an imidazopyridine structure. By an independent synthesis we were able to prove the structure **3** for Chatterjee's product **3**. He describes 2-(α-hydroxybenzyl-imidazo[4,5-c]pyridine as a compound with mp 330° and analytical data in agreement with the molecular formula. The spectroscopic data reveal, however, that the compound described is not the desired compound **8** but the isomeric pyridopyrazinone **3**.

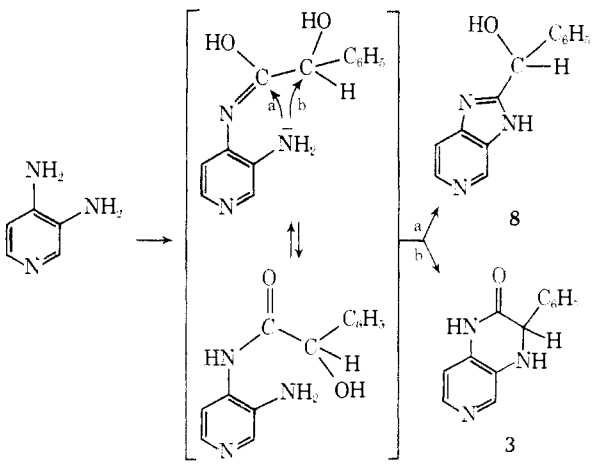
The still unknown imidazopyridine **8** was then prepared by unambiguous synthesis and showed to be an inhibitor that affects acid-sensitive viruses as a result of its ability to change the pH of the growth medium. The structures of both **3** and **8** were proved both spectroscopically and by independent synthesis.

The well-known procedure of Phillips[8] to prepare benzimidazoles in 4 *N* HCl is not applicable to the synthesis of imidazopyridines, as diaminopyridine exists in this medium exclusively as the hydrochloride and the proportion of free base necessary to initiate the reaction is lacking. It is more appropriate to employ other acids for catalysis or alternatively to accept a short detour *via* the benzyl (**6**) and benzoyl (**7**) compounds.

Synthesis of 2-Oxo-3-phenyl-1,2,3,4-tetrahydropyrido[2,3-c]pyrazine (3). In the reaction of mandelic acid with 3,4-diaminopyridine, ring closure can follow one of two paths, nucleophilic attack by the remaining free amino group either on the enolized amide bond with formation of the imidazopyridine (path a) or on the secondary hydroxyl group of mandelic acid to the pyridopyrazine (path b).

As initial amide formation could have occurred either at the C_3- or C_4-amino group of 3,4-diaminopyridine, two isomeric pyridopyrazinones **3** and **4** could result from the reaction. No differentiation was possible on the basis of the mass spectrum, as CO is eliminated from the compound in the primary fragmentation step, resulting in the formation of the same intermediate from each of the possible isomers. Solely, a weak positive nuclear Overhauser effect at the C_8 proton of the pyridine nucleus allowed tentative assignment of the correct structure **3** (Scheme I).

Scheme I



We decided finally to prove the assumed structure by unambiguous synthesis. 3-Fluoro-4-nitropyridine *N*-oxide (**1**)[9] was subjected to nucleophilic substitution with phenylglycinate (**2**). Catalytic hydrogenation over Raney nickel resulted in cyclization and reduction of the *N*-oxide in a single step to **3**. The spectra of **3** were identical with those of the compound prepared according to Chatterjee, *et al.*[7]

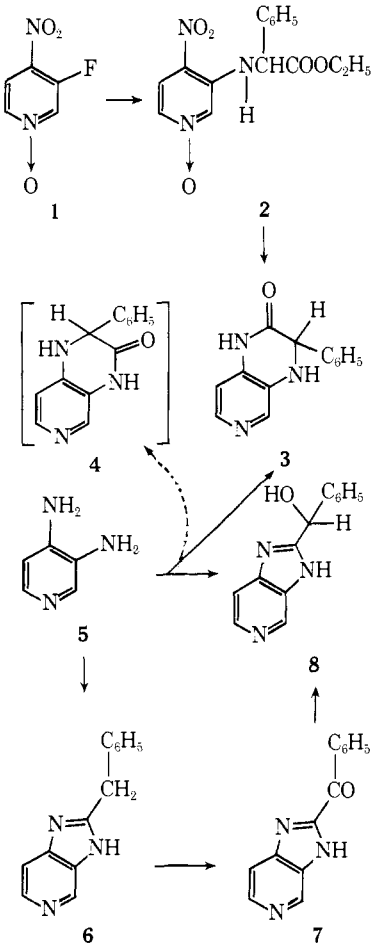
Synthesis of 2-(α-Hydroxybenzyl)imidazo[4,5-c]pyridine (8). 3,4-Diaminopyridine was allowed to react with the imido ethyl ester hydrochloride of phenylacetic acid to give 2-benzylimidazo[4,5-c]pyridine (**6**). The methylene group could be oxidized with SeO_2 to the carbonyl group **7**, which was then reduced with $NaBH_4$ to **8** (Scheme II).

Biological. Experiments to determine the antiviral activity of 2-(α-hydroxybenzyl)imidazo[4,5-c]pyridine were performed with various strains of poliovirus in HeLa cells and in the established line of African green monkey kidney cells BS-C-1.[10] In preliminary experiments it was found that the compound in concentrations up to 200 μg/ml caused no visually detectable alterations to the cells. An increasing acidification of the culture medium, consisting of Eagles[11] minimum essential medium fortified with 2% heat-inactivated fetal bovine serum, was noted.

The ability of the compound to inhibit the multiplication of polioviruses was tested *via* infectivity titrations of the viruses in the presence of 100 μg/ml of the compound and the results were compared with those obtained in the presence of 100 μg/ml of L-2-(α-hydroxybenzyl)benzimidazole (HBB).[12] When the attenuated polio vaccine strains

† H. Berner, *et al.*, unpublished results.

Scheme II



of Sabin[13] were used as test viruses, the compound inhibited viral multiplication to about the same extent as HBB.

To test whether this inhibition was due to a specific action of the compound or a consequence of the compound-induced acidification, the same experiments were repeated with a wild strain of poliovirus (Poliovirus Type I, Mahoney). Unlike the vaccine strains, the wild strains of poliomyelitis are capable to multiply at acid conditions.[14] This virus yielded in a plaque reduction test in the presence of 100 μg/ml of the compound the same number of plaques than found in the controls. The plaques, however, were small (3–5 mm) compared with the plaques in the controls (20–24 mm circular diameter). HBB, on the other hand, reduced the number of plaques by a factor of 10^4 and the few plaques found had a diameter of 10–15 mm.

On the basis of these findings it is concluded that 2-(α-hydroxybenzyl)imidazo[4,5-*c*]pyridine has no specific inhibitory activity on the multiplication of polioviruses. The increasing acidification of the culture medium containing the compound will completely inhibit the multiplication of the vaccine strains. However, the wild virus can multiply for a short period but is eventually also inhibited. This explains why the plaques produced by the Mahoney strain of poliovirus are reduced in size but not in number.

Experimental Section

Melting points (uncorrected) were taken on a Kofler hot-stage microscope. Proton nmr spectra were obtained on a Varian HA-100 instrument. TMS standard. Combustion analyses were in accord with the calculated percentages.

***N*-3-(4-Nitro-1-oxopyridyl)-α-phenylglycine Ethyl Ester (2).** 3-Fluoro-4-nitropyridine *N*-oxide[9] (5.4 g, 0.035 mol) was dissolved in 30 ml of benzene and treated with 12.3 g (0.07 mol) of phenyl glycine ester. The reaction mixture was stirred for 20 hr at 20° and the precipitate filtered and recrystallized from benzene: yield 9.8 g (94%); ir (KBr) 3350 (NH), 1735 (CO), 1575, 1330 (NO_2), 1243 cm^{-1} (N→O); uv (CH_3OH) 237 nm (ϵ 14,900), 326 (8530), 410 (5830); nmr ($CDCl_3$) 8.0 (d, 1 H, H_5, $J_{H(5)-H(6)}$ = 6 Hz), 7.6 (d, 1 H, H_2, $J_{H(2)-H(6)}$ = 2 Hz), 7.43 (dd, 1 H, H_6, $J_{H(6)-H(2)}$ = 2 Hz), 9.08 (d, 1 H, NH, J_{NH-CH} = 6 Hz), 7.4 (b, 5 H, C_6H_5), 5.08 (d, 1 H, -CHN, J_{CH-NH} = 6 Hz), 4.24 (dq, 2 H, $-C_2H_5$, J = 7 Hz), 1.24 (t, 3 H, $-C_2H_5$, J = 7 Hz). *Anal.* ($C_{15}H_{14}N_3O_5$) C, H, N.

2-Oxo-3-phenyl-1,2,3,4-tetrahydropyrido[2,3-*c*]pyrazine (3). 2 (50 mg, 0.00016 mol) was dissolved in 15 ml of methanol, 10 mg of Raney nickel and 0.1 ml of acetic acid were added, and the mixture was hydrogenated at 20°. After removal of the catalyst the filtrate was evaporated and the residue chromatographed on silica gel (eluent $CHCl_3$–MeOH, 7:1) and then recrystallized from methanol: yield 22 mg (60%); mp 330° dec; ir (KBr) 3320 (NH), 1690 cm^{-1} (CO); uv (CH_3OH) 225 nm (ϵ 42,300), 317 (3830); nmr (DMSO-$CDCl_3$) 8.02 (s, 1 H, H_5), 7.78 (d, 1 H, H_7, $J_{H(7)-H(8)}$ = 5 Hz), 6.78 (d, 1 H, H_8, $J_{H(8)-H(7)}$ = 5 Hz), 7.3 (s, 5 H, C_6H_5), 5.05 (d, 1 H, CHN, J_{CH-NH} = 2 Hz), 6.84 (b, 1 H, NH). *Anal.* ($C_{13}H_{11}N_3O$) C, H, N.

2-(α-Hydroxybenzyl)imidazo[4,5-*c*]pyridine (8). Method A. To a suspension of 550 mg (0.0025 mol) of 2-benzoylimidazo[4,5-*c*]pyridine (7) in 50 ml of 2-propanol was added 500 mg of $NaBH_4$ and the mixture stirred for 15 min at 20°. The reaction mixture was treated with saturated aqueous NaCl and repeatedly extracted with ethyl acetate. The combined extracts were dried over Na_2SO_4 and the solvent was removed *in vacuo*. Recrystallization from acetone–hexane gave a 420-mg (76%) yield: mp 141°; ir (KBr) 3500–2800 cm^{-1} (NH, OH); uv (CH_3OH) 243 nm (ϵ 5470), 249 (5590), 264 (6100); nmr (DMSO-$CDCl_3$) 8.82 (s, 1 H, H_7), 8.26 (d, 1 H, H_5, $J_{H(5)-H(4)}$ = 5 Hz), 7.4 (b, 6 H, H_4, $-C_6H_5$), 6.03 (s, 1 H, -CHOH). *Anal.* ($C_{13}H_{11}N_3O$) C, H, N.

Method B. 3,4-Diaminopyridine (1 g, 0.009 mol) and 1.5 g (0.01 mol) of mandelic acid were dissolved in 50 ml of xylene and the solution was boiled under reflux for 8 hr with separation of the water formed. The solvent was evaporated *in vacuo* and the residue chromatographed on silica gel (Kieselgel Merck; 0.05–0.2 mm; eluent $CHCl_3$–MeOH, 8:1). Fraction 1 gave 150 mg (6.7%) of pyridopyrazinone 3 and fraction 2 gave 185 mg (8.2%) of imidazopyridine 8.

2-Benzoylimidazo[4,5-*c*]pyridine (7). Benzylimidazo[4,5-*c*]pyridine (6, 1.2 g, 0.0057 mol) was dissolved in dioxane and boiled under reflux for 1 hr with 1 g of selenium dioxide. The reaction mixture was cooled and the red selenium filtered off. The ketone crystallized on standing and was recrystallized from propanol: yield 1 g (78%); mp 297°; ir (KBr) 1650 (CO), 2800–2400 cm^{-1} (NH); uv (CH_3OH) 238 nm (ϵ 6700), 292 (9925); nmr (DMSO-$CDCl_3$) 9.18 (s, 1 H, H_4), 8.5 (m, 2 H, H_6, H_7), 7.7 (m, 5 H, C_6H_5). *Anal.* ($C_{13}H_9N_3O$) C, H, N.

2-Benzylimidazo[4,5-*c*]pyridine (6). 3,4-Diaminopyridine (2 g, 0.018 mol) and 5.5 g (0.027 mol) of phenylacetimido ethyl ester hydrochloride were dissolved in 100 ml of ethanol and the solution was boiled under reflux for 8 hr. The reaction mixture was evaporated *in vacuo*, digested with water, and recrystallized from ethanol: yield 1.4 g (35%); mp 151°; uv (CH_3OH) 246 nm (ϵ 6820), 264 (7500); nmr ($CDCl_3$) 8.74 (d, 1 H, H_7, $J_{H(4)-H(7)}$ = 1 Hz), 7.38 (dd, 1 H, H_4, $J_{H(4)-H(5)}$ = 5.5 Hz), 8.2 (d, 1 H, H_5, $J_{H(5)-H(4)}$ = 5.5 Hz), 7.18 (s, 5 H, $-C_6H_5$), 4.3 (s, 2 H, $-CH_2$). *Anal.* ($C_{13}H_{11}N_3$) C, H, N.

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Pyrrole Antibacterial Agents. 1. Compounds Related to Pyoluteorin

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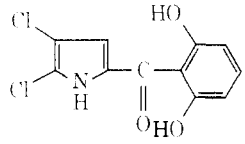
The naturally occurring halogenated pyrroles **1–5** have all yielded to synthesis.[1-9] All of these materials possess antibacterial properties of potential interest in human chemotherapy. We have prepared variations of pyoluteorin structure **1** and have examined them for *in vitro* and *in vivo* activity against a variety of pathogens and for antimalarial activity in mice. In addition, selected members of the series were examined for anthelmintic and antischistosomal activities.



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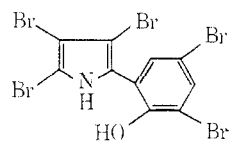

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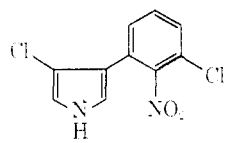

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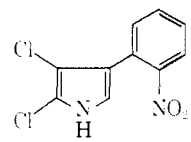

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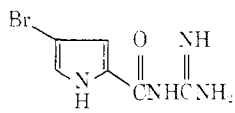


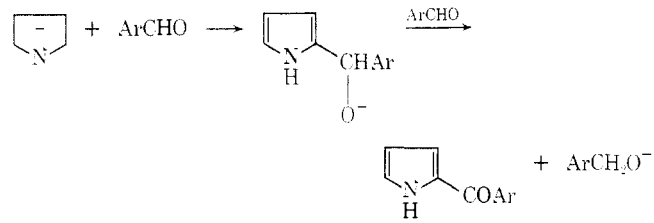
5

Chemistry. With the exception of **1** (see Experimental Section), three general methods were employed for the synthesis of 2-aroylpyrroles: acylation of pyrrole Grignard reagent[10] (method A), acylation with 4,5-dihalopyrrol-2-ylcarbonyl chloride (method B), and base-catalyzed condensation of pyrrole with arylaldehydes (method C).[11] The reagents for method B were conveniently prepared in high yield by halogenation of the trichloroacetylation product of pyrrole,[12] followed by hydrolysis and conversion of the resulting acid to the corresponding acyl chloride by means of $SOCl_2$.

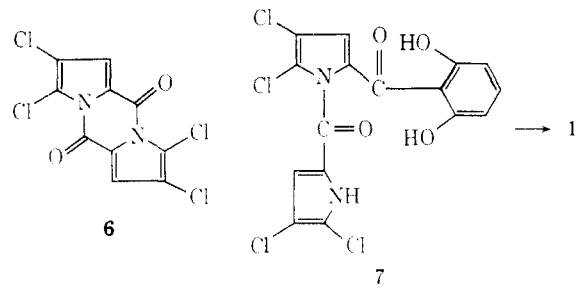
Method C has been described using equimolar quantities of $NaNH_2$, pyrrole, and arylaldehyde in refluxing C_6H_6 for 16 hr. The author reported that more than 1 equiv of aldehyde did not improve the yields (27–54%) despite the stoichiometry in the proposed mechanism (Scheme I). We have found that the product 2-aroylpyrroles undergo proton exchange with the pyrrolylsodium† and thus consume part of the limiting reagent. Using excess aldehyde with pyrrole and NaH in a molar ratio of 1:2 and with a 10% solution of DMF in C_6H_6 as solvent, yields of 70–74% were obtained in 1–3 hr. Halogenation of the 2-aroylpyrroles was carried out using the element in HOAc.[14] Cleavage of methyl ethers was accomplished using $AlCl_3$ or $AlBr_3$. Pyoluteorin itself was prepared from the pyrocoll **6** and 1-lithio-2,6-di(tetrahydropyranyloxy)-benzene,[15] followed first by acid hydrolysis (giving **7**) and then base hydrolysis (Scheme II). The compounds prepared by the above procedures are listed in Table I.

†For a discussion of acidity of substituted pyrroles, see ref 13.

Scheme I



Scheme II



6 **7** **1**

Biological Screening. All compounds with an *in vitro* MIC of 15.6 μg/ml or less against *Staphylococcus aureus* are shown in Table II along with their activity against three other organisms.

Although some of these compounds (**1**, **14**, **18**, **27**, **31**) showed a high order of *in vitro* activity against *Staph. aureus* at 200 mg/kg sc, none was effective in preventing mortality in mice infected with this organism or with *Klebsiella pneumoniae*. All compounds, when given at 200 mg/kg po, were ineffective in reducing parasite counts in mice infected with *Plasmodium berghei* (NK65 or NYU-2 strains). Selected agents at a dose of 100–200 mg/kg po did not reduce worm populations in mice infected with *Schistosoma mansoni* (**9**, **12**, **15–17**, **22**, **30**, **31**) nor produce clearances in mice infested with pinworms, roundworms, or tapeworms (**11–14**, **16**, **20**, **26**, **28**, **29**, **32**). Bailey and Rees[1] have reported that pyoluteorin has *in vitro* activity comparable to or better than cryptosporiopsin or nystatin against the Dutch elm disease fungus *Ceratocystis ulmi*. Agar growth studies‡ with this organism revealed that the isomers of pyoluteorin **26** and **30** were respectively 5 and 14 times as potent as **1** in inhibiting the growth of the fungus. Furthermore, the nonhydroxylated analog **18** was 20 times as effective as **1** (all drugs at 10 ppm).

Experimental Section§

Aryl 2-Pyrrolyl Ketones. General syntheses for compounds in Table I are as follows.

Method A. The procedure of Pesson, *et al.*,[10] was followed exactly.

‡We are grateful to Mr. O. F. Greenwell and Mr. J. R. Willard of the Niagara Chemical Division of FMC Corp., Middleport, N. Y., for providing us with the *in vitro* data.

§All melting points were obtained on a Mel-Temp apparatus and are uncorrected. Where glpc analyses were used, determinations were performed on a Hewlett-Packard research chromatograph, Model 5751B, equipped with glass columns packed with 3% OV 17 on 100–120 mesh Gas Chrom Q. Microanalytical determinations were carried out by Instranal Laboratories, Inc., Rensselaer, N. Y., and Galbraith Laboratories, Inc., Knoxville, Tenn.