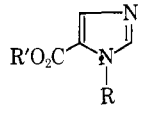
Antifungal Properties.—The fungistatic assay was carried out in Sabouraud's liquid (1 g of neopeptone Difco and 2 g of glucose Difco per 100 ml of distilled water) in 16 × 160 mm test tubes, each containing 4.5 ml of liquid medium which had been autoclaved at 120° for 15 min. The compounds to be tested were dissolved in 50% alcohol at initial concentration of 20 mg/ml. The solutions were subsequently diluted with sterile distilled water to give a concentration of 10 mg/ml. Successive decimal dilutions were made in distilled water. To tubes containing 4.5 ml of Sabouraud's liquid medium 0.5 ml of the solution of the drug was added, thereby obtaining concentrations of 1000, 500, 100, 10, and 1 μg/ml of medium. Control tubes were prepared by adding 0.5 ml of distilled water to 4.5 ml of medium, alcohol being added to give concentrations identical with the tubes containing 1000 and 500 μg of the drug. The filamentous fungi were incubated in Sabouraud's agar at 25° for 2–3 weeks. A block of 2 × 2 × 2 mm was then inoculated into the medium. All cultures were made in duplicate and were incubated at 25° for 14 days. Readings were then taken and were expressed as + if inhibition at the 100-μg/ml level was complete and as ++ if total inhibition occurred at 10 μg/ml.

Results

The results are summarized in Table II. Clearly, compounds of type IIIa,b exhibit excellent fungistatic activity against several organisms. Optimum effect is achieved with lower esters **6** and **9**. Lengthening of the chain results in diminished activity, notably against *Microsporum canis*. Chain interruption, in one case, (**13** *vs.* **17**) increases activity. The introduction of bulky groups causes greatly diminished inhibition (*i.e.*, **18**–**20**), while carboxylic acids **3** and **7** are totally inactive.

TABLE II
ANTIFUNGAL ACTIVITIES



Compd	R	R′	Lowest level of inhibition[a] *M. canis*	*T. mentagrophytes*	*T. rubrum*
2	1-Indanyl	CH_3	+	+	+
4	1-Indanyl	C_2H_5	+	++	++
8	1-Tetralyl	CH_3	+	+	++
6	1-Tetralyl	C_2H_5	++	++	++
9	1-Tetralyl	n-C_3H_7	++	++	++
10	1-Tetralyl	i-C_3H_7	+	+	+
11	1-Tetralyl	n-C_4H_9	+	++	++
12	1-Tetralyl	i-C_4H_9	+	++	++
13	1-Tetralyl	n-C_8H_{17}	0	+	+
14	1-Tetralyl	$CH_3CHCH_2CH(CH_3)_2$	0	0	0
15	1-Tetralyl	$CH_2CH{=}CH_2$	+	++	++
16	1-Tetralyl	$CH_2CH_2OCH_3$	+	++	++
17	1-Tetralyl	$CH_2CH_2OC_2H_5$	+	+	++
18	1-Tetralyl		+	+	+
19	1-Tetralyl	C_6H_{11}	0	0	++
20	1-Tetralyl	$CH_2C_6H_5$	0	0	++

[a] Total inhibition at 100 μg, +; at 10 μg, ++.

For comparative purposes sodium undecalenate, diamthazole,[3] and chlormidazole[4] were assayed concurrently against *M. canis*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*. None of these caused total inhibition below the 100-μg/ml level. One of our compounds, 1-(1-tetralyl)imidazole-5-carboxylic acid ethyl ester (**6**, proposed generic name, ethonamidate) has been selected for clinical evaluation. A more detailed pharmacological study of ethonamidate will be presented elsewhere.[5]

Acknowledgment.—We are indebted to Mr. J. Dockx for the preparation of starting materials and to Messrs. A. Sels and W. Verkest for the analytical determinations reported herein. Financial support of the Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw is gratefully acknowledged.

(3) Asterol® (Roche).
(4) Polycid® (Grünenthal GmbH).
(5) R. Vanbreuseghem, J. Van Cutsem, and D. Thienpont, *Chemotherapia*, in press.

Lincomycin. VII. 4′-Depropyl-4′-ethoxylincomycins

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Variation in the length of the 4′-alkyl substituent of the antibiotic lincomycin (**1**) produced a series of analogs, some of which possessed enhanced antibacterial activity.[1] This note describes the synthesis and antibacterial activity of 4′-depropyl-4′-ethoxylincomycin (**3**) and its *cis* isomer **4**. These compounds may be classified as classical bioisosteres[2] of lincomycin and its *cis* isomer **2** in which the methylene adjacent to the proline ring in the 4′-propyl substituent is replaced by oxygen.

1-Carbobenzoxy-4-hydroxy-L-proline[3] was converted to the benzyl ester (**6**) and the latter etherified by the excellent method of Kuhn,[4] to form crude **7** in almost quantitative yield. Hydrogenolysis of **7** afforded 4-ethoxy-L-proline (**8**) as well as a small amount of the diketopiperazine **15**. Reductive methylation of **8** proceeded smoothly yielding **9**. Condensation of 1-methyl-4-ethoxy-L-proline (**9**) with methyl thiolincosaminide[5] using the mixed-anhydride procedure led to crystalline 4′-depropyl-4′-ethoxylincomycin (**3**).

In a similar manner, 1-carbobenzoxy-4-*cis*-hydroxy-L-proline[3] was converted to 1-methyl-4-*cis*-ethoxy-L-proline (**14**) and then to 4′-depropyl-4′-*cis*-ethoxylincomycin (**4**). In **4** the ethoxy substituent at C-4′ is oriented *cis* to the L-amide group, the same configuration as *cis*-lincomycin.[1]

4′-Depropyl-4′-ethoxylincomycin (**3**) and 4′-depropyl-4′-*cis*-ethoxylincomycin (**4**) have about 2% the activity of lincomycin when assayed in the standard-curve assay against *Sarcina lutea*.[6] Both compounds were inactive when administered subcutaneously at

(1) B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *J. Med. Chem.*, **10**, 355 (1967).
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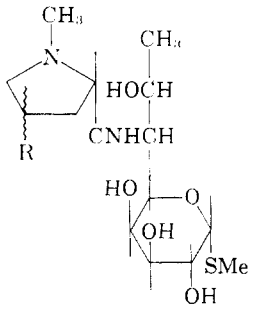
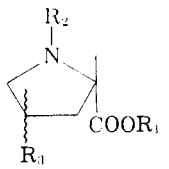
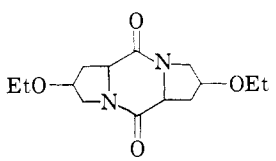
(6) L. J. Hanka, D. J. Mason, M. R. Burch, and R. W. Treick in "Antimicrobial Agents and Chemotherapy—1962," J. C. Sylvester, Ed., American Society for Microbiology, Ann Arbor, Mich., 1963, p 565.



1, **3**, R = *trans*
2, **4**, R = *cis*
1, **2**, R = $CH_2CH_2CH_3$
3, **4**, R = OC_2H_5



5–**9**, R_3 = *trans*
10–**14**, R_3 = *cis*
5, **10**, R_1 = H; R_2 = Cbz; R_3 = OH
6, **11**, R_1 = $C_6H_5CH_2$; R_2 = Cbz; R_3 = OH
7, **12**, R_1 = $C_6H_5CH_2$; R_2 = Cbz; R_3 = OC_2H_5
8, **13**, R_1 = H; R_2 = H; R_3 = OC_2H_5
9, **14**, R_1 = H; R_2 = CH_3; R_3 = OC_2H_5



15

320 mg/kg and orally at 800 mg/kg to mice infected with *Staphylococcus aureus*. Lincomycin, run as a control, protected at 13 mg/kg when administered subcutaneously and at 49 mg/kg orally.[7]

Experimental Section[8]

1-Carbobenzoxy-4-ethoxy-L-proline Benzyl Ester (7).—A mixture of 219 g of 1-carbobenzoxy-4-hydroxy-L-proline (**5**),[3] 80 ml of benzyl alcohol, 300 ml of benzene, and 1 g of *p*-toluenesulfonic acid was heated under reflux and the distillate was passed through a Dean–Stark distillation head. During 17 hr, an additional 400 mg of *p*-toluenesulfonic acid and 100 ml of benzene were added. The reaction mixture was separated into neutral and acidic fractions by partition between $NaHCO_3$ solution and benzene. The oily, neutral fraction weighed 219 g. Thin layer chromatography using a cyclohexane–acetone system (1:1) showed one spot at R_f 0.69 when sprayed with 0.1 *N* aqueous I_2 solution.

The crude ester from above was dissolved in 750 ml of dimethylformamide (DMF) and 372 g of EtI. Silver oxide (462 g) was added, and the mixture was stirred for 3 days at ambient temperature then filtered, and the residue was washed successively with DMF and CH_2Cl_2. The solvent was distilled under vacuum. The residue was dissolved in CH_2Cl_2. The solution was washed with H_2O several times and dried, and the solvent was distilled. The resulting oil, 223 g, showed one spot on tlc run as described above (R_f 0.76).

4-Ethoxy-L-proline (8) and 2,7-Diethoxyoctahydro-5H,10H-dipyrrolo[1,2-*a*:1,2′-*d*]pyrazine-5,10-dione (15).—Compound **7** (17 g) in 200 ml of MeOH containing 2 g of 10% Pd–C was shaken under H_2 for 18 hr. The catalyst was removed by filtration. The solvent was evaporated and the residue was crystallized from methanol–ether to yield 2.9 g of **8**, mp 185–188°. The mother liquor was evaporated and the residue was dissolved in 150 ml of MeOH and rehydrogenated over 2 g of catalyst. An additional 2.2 g of **8**, mp 194–198°, was thus obtained. An analytical sample was prepared by two recrystallizations from methanol–ether. It melted at 194–198° and showed one spot on tlc [methanol–NH_4OH (17%) 9.5:0.5; detection by ninhydrin]; $[\alpha]_D$ −46° (H_2O).

Anal. Calcd for $C_7H_{13}NO_3$: C, 52.81; H, 8.23; N, 8.80. Found: C, 52.63; H, 8.46; N, 8.77.

The mother liquors from some runs yielded a more soluble compound which was purified by recrystallization from ethyl acetate–Skellysolve B. Infrared analysis indicated the diketopiperazine **15**, mp 123–125°.

Anal. Calcd for $C_{12}H_{22}N_2O_4$: C, 59.55; H, 7.85; N, 9.92. Found: C, 59.46; H, 7.92; N, 9.83.

1-Methyl-4-ethoxy-L-proline (9).—A mixture of 3.75 g of **8**, 300 mg of 10% Pd–C, 3.5 ml of formalin, and 150 ml of MeOH was shaken under H_2 for 4 hr. The catalyst was removed by filtration and the solvent was evaporated *in vacuo*. Benzene was added to the residue and then evaporated *in vacuo* to yield a crystalline residue, ninhydrin test negative. The crude crystals were dissolved in MeOH, and the solution was clarified by filtration; crystals precipitated by the addition of ether. The yield of **9**, mp 109–113°, was 2.7 g. The analytical sample, mp 117–119°, $[\alpha]_D$ −68° (H_2O), was prepared by several recrystallizations from ethyl acetate.

Anal. Calcd for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.39; H, 8.85; N, 8.08.

4′-Depropyl-4′-ethoxylincomycin (3).—To a solution of 5.2 g of 1-methyl-4-ethoxy-L-proline (**9**) and 4.9 ml of Et_3N in 200 ml of acetonitrile cooled to 0° was added 4.1 ml of isobutyl chloroformate. The reaction mixture was stirred for 0.25 hr, and a solution of 10.95 g of methyl thiolincosaminide in 150 ml of water was added. The mixture was stirred at 0° for 1 hr and at ambient temperature for 7 hr. The acetonitrile was evaporated under vacuum. The residue was diluted with water and treated with two 30-g portions of Dowex 2 (OH form). The solution was lyophilized. The dried residue was shaken with 500 ml of acetone and filtered. The acetone solution was acidified with dilute HCl, precipitating crude **3**·HCl as a gum which crystallized on standing. It was recrystallized by dissolving in water and adding acetone. The yield of **3**·HCl was 7.79 g (56.6%), mp 150–160° (solvated). Similar material on recrystallization afforded a hydrate, mp 170–175° dec, $[\alpha]_D$ −145° (H_2O).

Anal. Calcd for $C_{17}H_{32}N_2O_5$: C, 45.88; H, 7.48; N, 6.30; S, 7.21. Found: C, 45.84; H, 7.74; N, 6.05; S, 7.27 (corrected for 2.53% H_2O, Karl-Fischer titration).

1-Carbobenzoxy-4-*cis*-ethoxy-L-proline Benzyl Ester (12). 1-Carbobenzoxy-4-allohydroxy-L-proline (**10**)[3] (10 g) was esterified and ethylated as described for the preparation of **7**. The crude product was chromatographed over 400 g of Florisil using Skellysolve B (bp 60–71°) with increasing amounts of acetone for elution. An oily fraction showing one spot by tlc (R_f 0.74) was obtained and used directly in the following step.

4-*cis*-Ethoxy-L-proline (13).—In the manner described above for the preparation of **8**, 3.4 g of **12** was hydrogenated to yield a total of 67.5% of **13**, mp 185–205°, $[\alpha]_D$ −36° (H_2O). This material was difficult to crystallize, forming gels very readily. The melting point of the crystals obtained varied considerably, some melting as high as 214–216°.

Anal. Calcd for $C_7H_{13}NO_3$: N, 8.80. Found: N, 8.44.

1-Methyl-4-*cis*-ethoxy-L-proline (14).—In the manner described for the preparation of **9**, 1 g of **13** was converted to **14** (900 mg), mp 163–165° (from MeOH–EtOAc), $[\alpha]_D$ −61° (H_2O).

Anal. Calcd for $C_8H_{15}NO_3$: C, 55.46; H, 8.73; N, 8.09. Found: C, 56.05; H, 9.02; N, 8.10.

A portion was converted to the hydrochloride which was recrystallized from methanol–ether. It melted at 141–143°.

Anal. Calcd for $C_8H_{15}NO_3$·HCl: C, 45.82; H, 7.69; N, 6.69. Found: C, 46.00; H, 7.52; N, 7.01.

4′-Depropyl-4′-*cis*-alloethoxylincomycin (14).—As previously described for the preparation of **3**, 0.52 g of **14** was treated with 1.09 g of methyl thiolincosaminide to yield 0.77 g (57.6%) of **4**,

(7) C. Lewis, H. W. Clapp, and J. E. Grady, ref 6, p 570.

(8) Melting points were taken in a Thomas–Hoover Unimelt apparatus and were corrected for stem exposure. Thin layer chromatography (tlc) was carried out on microslides coated with Brinkmann silica gel GF_{254}. Optical rotations were observed at 26° at $c \sim 1$. Infrared and nmr data are available on request.

mp 146–152°. A portion was recrystallized from acetone–water. It melted at 141–146° and had $[\alpha]_D$ +132° (H_2O).

Anal. Calcd for $C_{17}H_{32}N_2O_7S \cdot HCl$: C, 45.88; H, 7.48; N, 6.30; S, 7.21. Found: C, 45.39; H, 7.60; N, 6.41; S, 7.09 (corrected for 4.64% water, Karl-Fischer titration).

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Bis(pyrimidine nucleoside) Phosphates[1]

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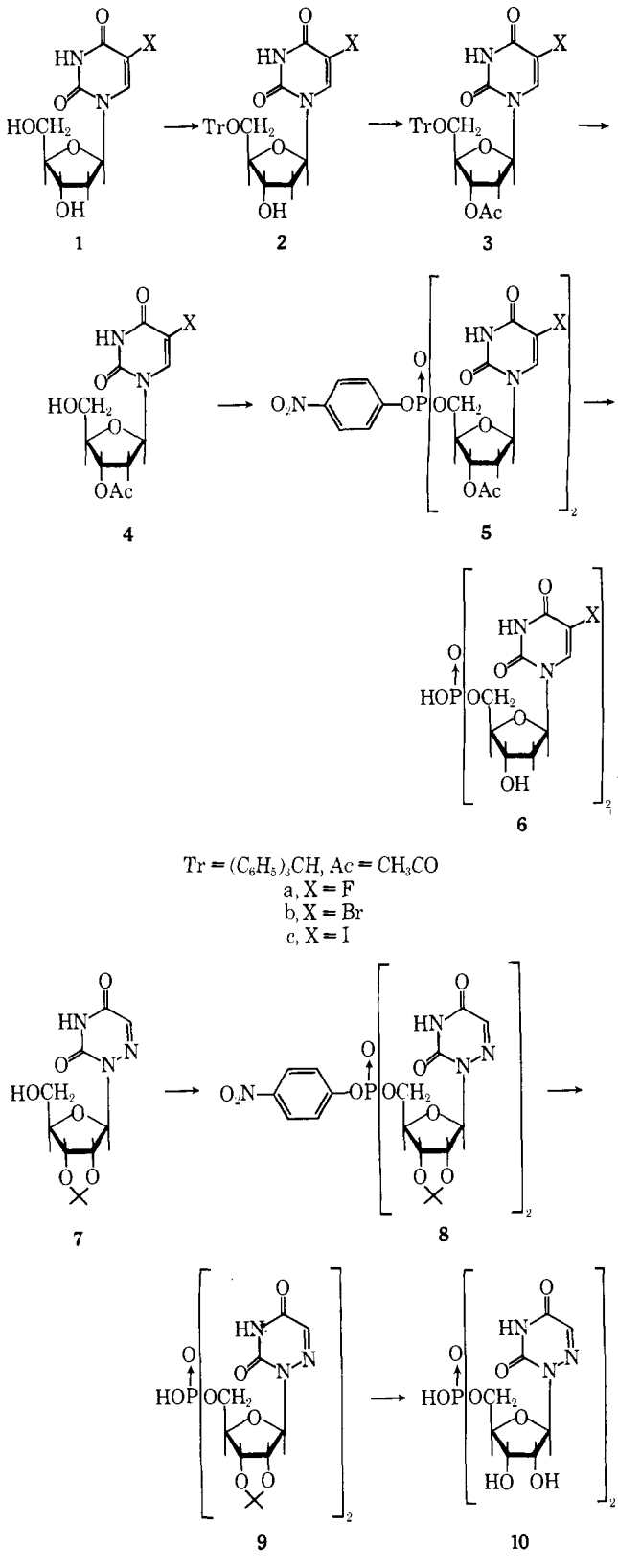
6-Mercaptopurine must be metabolized to its ribonucleotide before it can inhibit the growth of cells.[2] Cells that lack the IMP–GMP pyrophosphorylase necessary for this conversion are resistant to 6-mercaptopurine,[2] and to its ribonucleotide,[3] which is unable to enter cells intact.[4] In an effort to overcome this problem of resistance, a number of derivatives of 6-mercaptopurine ribonucleotide that might enter cells were synthesized.[5] One of these, thioinosinyl-(5′→5′)-thioinosine,[5] was found to be cytotoxic to a subline of HEp-2 cells resistant to 6-mercaptopurine.[3] This observation led us to synthesize the same type of derivative of the biologically active pyrimidine nucleoside analogs (**1a–c** and 6-azauridine). The same general approach used in our previous work[5] was employed. 5-Bromo-2′-deoxyuridine (**1b**) and 2′-deoxy-5-iodouridine (**1c**) were converted to their 5′-*O*-trityl derivatives (**2b** and **c**) for acetylation. Removal of the trityl group from the acetylated nucleosides (**3b** and **c**) gave nucleosides (**4b** and **c**) suitably blocked for conversion to the desired phosphates. The preparation of 3′-*O*-acetyl-2′-deoxy-5-fluorouridine (**4a**) was described previously.[5] 6-Azauridine was converted to its 2′,3′-*O*-isopropylidene derivative (**7**).[6] These blocked nucleosides (**4** and **7**) were then allowed to react with *p*-nitrophenylphosphorodichloridate[7] and the blocked nucleotides (**5**) were treated with base to remove the acetyl group and the *p*-nitrophenyl group to give the desired 2′-deoxy-5-halouridylyl-(5′→5′)-2′-deoxy-5-halouridines (**6**).[8,9] Treatment of the azauridine derivative (**8**) with base removed the *p*-nitrophenyl group, but removal of the 2′,3′-*O*-isopropylidene group was easily accomplished by simply refluxing an aqueous solution of **9** which, in itself, is acidic enough to effect the hydrolysis to **10** (see Scheme I).

SCHEME I



Biologic Data.—The cytotoxic effect of these dinucleoside phosphates on KB cells is compared to the effect of the corresponding nucleosides in Table I. The cells were grown on glass and growth was measured by determination of protein content.[10]

(1) This investigation was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH43-64-51.

(2) R. W. Brockman, *Advan. Cancer Res.*, **7**, 129 (1963).

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(8) The synthesis of 2′-deoxy-5-fluorouridylyl-(5′→5′)-2′-deoxy-5-fluorouridine by another method has been reported.[9]

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(10) V. I. Oyama and H. Eagle, *Proc. Soc. Exptl. Biol. Med.*, **91**, 305 (1956); *Cancer Chemotherapy Rept.*, **25**, 57 (1962).