An Improved, Large-Scale Synthesis of Xanthothricin and Reumycin [1]

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Xanthothricin (toxoflavin) has been synthesized in high yield and on a large (over 100 g) scale; this was selectively demethylated at N-1 to produce reumycin (1-demethyltoxoflavin) employing an optimized purification protocol also amenable to large-scale production.

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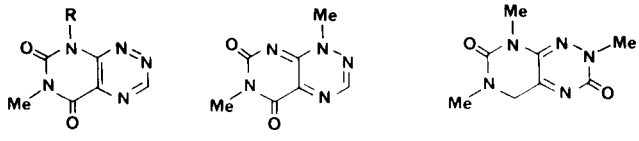
Reumycin (1-demethyltoxoflavin, **1**), although itself not found in nature, possesses the pyrimido[5,4-*e*]-1,2,4-triazene (7-azapteridine) ring system common to several naturally-occurring analogs which exhibit considerable antibiotic and other useful pharmacological activity [2]. Thus, much effort has been expended in the development of useful synthetic routes toward this class of compounds, which includes fervenulin (**1a**) [3], xanthothricin (toxoflavin, **2**) [4], and 2-methylfervenulone (**3**) [5].



1 (R = H)
1a (R = Me)

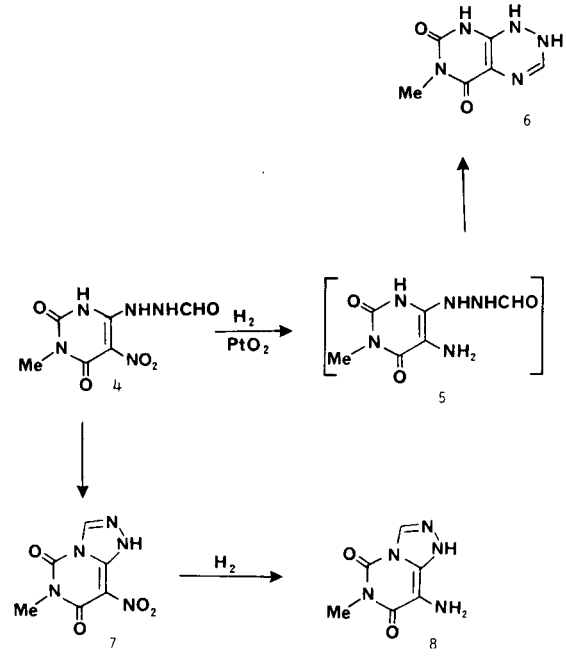
2

3

In 1966, Cheng and coworkers reported a most elegant preparation of reumycin on a small scale [6]. Initial testing by the National Cancer Institute indicated significant activity against B16 melanocarcinoma in mice; the compound has since been shown to be effective also against carcinosarcoma (but not leukemia) [7]. We thus required large (*ca.* 30-50 gram) quantities of pure material for further study and biological evaluation.

Although the reported synthesis performed admirably on a gram scale, attempts to employ the route on the large scale necessary for our purposes failed in our hands. The key step of the route involved the *in situ* cyclization of amino formylhydrazino uracil **5**, derived from the corresponding nitro compound **4**. During this transformation, a major side product **8** was reportedly formed *via* cyclization of **4** toward the ring nitrogen atom prior to reduction of the nitro functionality to afford the triazolo[4,3-*c*]pyrimidine **7**, which then underwent hydrogenation to produce **8** as the isolated byproduct. This sequence is depicted in Scheme I. Unfortunately, we found that on a large scale *only* **6** was produced despite numerous adjustments of many reaction variables. Evidently, the longer reaction times necessary to effect complete reduction of the nitro group in **4** on a larger scale allow sufficient time for essentially complete reaction with the ring nitrogen atom. Additionally, the exothermicity of most catalytic hydrogenations [8] can often result in localized heating during large-scale reactions, despite vigorous agitation of the reaction vessel. Increasing the hydrogen gas pressure served only to aggravate this problem. Variation of the catalyst, including a gradual decrease in the metal loading on a carbon support, inevitably resulted in extended reaction times, with similar consequences. Although small amounts of the desired product were isolable *via* chromatography, we discarded this approach as untenable for a large-scale synthesis of the required ring system.

Scheme I

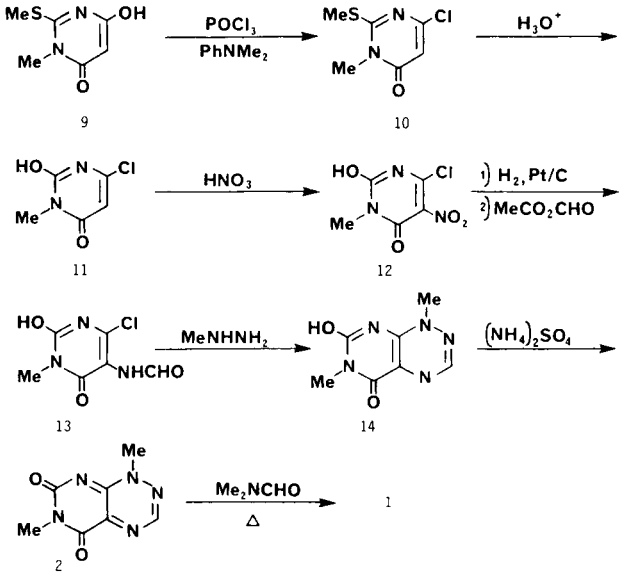


In 1973, Japanese workers discovered that xanthothricin (**2**) and several analogs could be selectively demethylated at N-1 by nucleophilic solvents such as dimethylformamide [9c,d]; this technique was later refined to employ secondary amines (*e.g.*, diethylamine) as the nucleophile [10]. This ingenious approach seemed well-suited

to the production of reumycin, requiring only an efficient synthesis of xanthothricin (which is produced commercially from cultures of *Streptomyces brunneus xanthothricini* [11]). Although a synthesis of xanthothricin was described, its low yield and obvious unscalability (in that a separation *via* preparative thin-layer chromatography was required) led us to seek other approaches.

We describe herein an efficient route to reumycin, based upon significant chemical and logistical improvements of the early syntheses of xanthothricin [3,8] coupled with experimental optimization of the demethylation procedure. The overall sequence is depicted in Scheme II.

Scheme II



6-Hydroxy-3-methyl-3-methylthio-4(3*H*)-pyrimidinone (**9**) [3a] was chlorinated by the *slow* addition of *N,N*-dimethylaniline to a slurry of **9** in phosphorus oxychloride. Acidic hydrolysis of the resulting chloro derivative **10** afforded dione **11** in 54% yield, based upon recovered starting material (which was recycled). Low temperature mixed-acid nitration produced the key chloro nitro derivative **12** in a high state of purity.

Reduction of the nitro group was effected *via* catalytic hydrogenation in alkaline aqueous methanol over platinum-on-carbon, which we found to give superior results to those obtained with the previously reported, more expensive platinum oxide. The crude, extremely air-sensitive amine product was immediately treated with formic acetic anhydride [12] to afford the formamide derivative **13** in 63% overall yield from **12**. On our 50-gram scale, we found it crucial to perform the latter step with dry ice-acetone bath cooling to control the violent exotherm.

The formation of the triazine ring entailed the reaction of formamide **13** with methylhydrazine, which smoothly displaced the ring chlorine atom, followed by spontaneous cyclization through condensation with the aldehyde functionality of the adjacent formamide. The crude product was then dehydrogenated to xanthothricin (**2**) employing ammonium sulfate. In contrast to the original report [6], we found the oxidation of the cyclized intermediate **14** to be very slow and temperature-sensitive. Increasing the reaction temperature resulted in molecular decomposition, necessitating long reaction times at ambient temperature and recycling of recovered **14** in order to achieve acceptable and reproducible yields.

The demethylation, carried out as described [8], failed to afford crystalline reumycin (**1**). Xanthothricin (**2**) was refluxed in dimethylformamide for several hours, whereupon solvent removal afforded a tarry brown residue which thwarted all efforts at purification *via* recrystallization or trituration. We obtained extremely pure material, however, by adsorption of the sticky product on Celite, Soxhlet extraction with ether, and filtration through silica gel.

In summary, we have developed a much-improved route to reumycin, featuring a synthesis of xanthothricin in far greater yield than any previously reported, coupled with optimization of a unique *N*-demethylation procedure amenable to the production of at least 30-40 gram quantities of analytically pure material.

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. The ir spectra were obtained on a Nicolet FT-3600 infrared spectrophotometer; absorption maxima are reported in wavenumbers (cm^{-1}). The nmr signals were recorded on a Hitachi Perkin-Elmer R-24B spectrometer and are relative to tetramethylsilane internal standard. Chemical shifts are reported downfield from tetramethylsilane (TMS) in parts per million of the applied field. Peak multiplicities are abbreviated as follows: singlet, s; doublet, d; triplet, t; quartet, q; multiplet, m; envelope, e. Thin-layer chromatographic analyses were carried out on Analtech silica gel "G" plates using the specified solvent as eluent; visualization was effected by either ultraviolet light or by charring with phosphomolybdic acid. Elemental analyses were performed either by Micro-Tech, Skokie, IL or by the Quality Control Department of the Aldrich Chemical Company.

6-Chloro-3-methyl-2-methylthio-4(3*H*)-pyrimidinone (**10**).

A slurry of 6-hydroxy-3-methyl-2-methylthio-4(3*H*)-pyrimidinone [3a] (**9**, 3500 g, 26.3 moles) in phosphorus oxychloride (10.3 l) was stirred at 55° as *N,N*-dimethylaniline (1500 ml) was added over a 30 minute period, causing the mixture to reflux. After being heated at reflux for one hour, the mixture was stripped of excess phosphorus oxychloride *in vacuo* and the residual brown oil was poured into ice water (40 l) with stirring. The product was separated by filtration, washed with water until the washings were colorless, air-dried, and recrystallized from heptane (including charcoal decolorization) to afford **10** (1965 g, 51%) as yellow-orange crystals, mp 113-114.5° (lit [3a] 111-112°).

6-Chloro-2-hydroxy-3-methyl-4(3*H*-pyrimidinone (**11**).

A mixture of 50% aqueous ethanol (5 l), concentrated hydrochloric acid (750 ml), and 6-chloro-3-methyl-2-methylthio-4(3*H*)-pyrimidinone (**10**, 500 g, 2.62 moles) was heated at reflux for 14 hours, the resulting solution evaporated to dryness, and the solid residue stirred in ether (2.5 l)

for 2 hours. The solid was collected by filtration and air-dried. The ether was washed with 10% aqueous sodium hydroxide solution, then water and brine, dried over anhydrous sodium sulfate, and concentrated to afford pure starting material (104 g, 21% recovery). The solid collected by filtration was recrystallized from ethanol (4 l) to give pure **11** (180 g, 54% based on consumed starting material), mp 282-283° dec, (lit [3a] 277-279° dec).

6-Chloro-2-hydroxy-3-methyl-5-nitro-4(3*H*)-pyrimidinone (**12**).

6-Chloro-2-hydroxy-3-methyl-4(3*H*)-pyrimidinone (**11**, 360 g, 2.2 moles) was dissolved portionwise in concentrated sulfuric acid, keeping the temperature below 15°. Fuming nitric acid (360 ml) was added dropwise over a one hour period, still maintaining the temperature below 15°, whereupon the reaction mixture was allowed to return to room temperature. After an additional 30 minutes, the mixture was poured onto ice (*ca.* 1 kg) with stirring. When the ice had melted the product was collected by suction filtration, washed with cold water (2 × 1.5 l), and dried (25°/16 hr/110 mm; then 25°/24 hours/0.05 mm) to afford **12** as a pale yellow powder (320 g, 69%), mp 190-193° dec, (lit [3a] 190-193°).

6-Chloro-5-formamido-2-hydroxy-3-methyl-4(3*H*)-pyrimidinone (**13**).

A 2-l Paar bottle was charged with 5% platinum-on-carbon (5 g). The bottle was purged with argon while methanol (200 ml) was slowly added [13]. Additional methanol (1300 ml) was added, followed by 6-chloro-2-hydroxy-3-methyl-5-nitro-4(3*H*)-pyrimidinone (**12**, 50 g, 0.24 mole) and finally concentrated ammonium hydroxide (50 ml). The resulting mixture was hydrogenated (10-45 psi) until hydrogen uptake ceased (13.2 l, 82% of theoretical). The almost colorless reaction mixture was filtered through Celite under a stream of nitrogen to give a clear, deep burgundy liquid which was concentrated *in vacuo* (10 mm, <25°) to leave a red solid. Methylene chloride (100 ml) was added, followed by the *slow* (the reaction is *strongly* exothermic) addition of formic acetic anhydride [12] (100 ml) with cooling in a dry ice-acetone bath as needed to keep the temperature below 40°. The solid material dissolved, shortly after which time a brown precipitate appeared. After 15 minutes, ethanol (500 ml) was added and the solid was collected by filtration, washed with ethanol (150 ml) and ether (3 × 200 ml), and air-dried to give **11** as a light-tan solid (30.9 g, 63%), mp 225-226°. An analytical sample was secured *via* two recrystallizations from dioxane, mp 228-230° (lit [3a] 225-226°); ir (potassium bromide): 3440 (OH), 3274 (NH), 1717 (C=O), 1647 (NHC=O) cm^{-1}; nmr (deuteriochloroform/DMSO-d_6): 12.23 (br s, 1H, O*H*), 9.18-8.62 (m, 1H, N*H*), 8.07-7.62 (m, 1H, C*H*O), 3.10 (s, 3H, CH_3).

Anal. Calcd. for $C_6H_6ClN_3O_3$ (203.58): C, 35.40; H, 2.97; N, 20.64. Found: C, 35.31; H, 3.00; N, 20.46.

1,6-Dimethylpyrimido[5,4-*e*]-1,2,4-triazine-5,7(1*H*,6*H*)-dione (Xanthothricin, **2**) [14].

A mixture of ethanol (4850 ml), 6-chloro-5-formamido-2-hydroxy-3-methyl-4(3*H*)-pyrimidinone (**13**, 242.5 g, 1.2 moles), and methylhydrazine (63.4 ml, 1.2 moles) was heated to reflux with stirring for 2.5 hours, cooled to room temperature, and the resulting red-brown solid **14** was collected by filtration. This material was stirred in saturated ammonium sulfate solution (6.5 l) for two days, the mixture was filtered, and the recovered red-brown solid was stirred in additional saturated ammonium sulfate solution (6.5 l). Meanwhile, the bright yellow filtrate was continuously extracted with chloroform (5 l) until the chloroform layer in the extractor was almost colorless. This procedure was carried out four times until all of the red-brown material had been consumed. The chloroform extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to afford xanthothricin (**2**, 124 g, 41%), mp 178-180° dec, (lit [3a] 172-173° dec).

6-Methylpyrimido[5,4-*e*]-1,2,4-triazine-5,7[6*H*,8*H*]-dione (Reumycin, **1**).

A mixture of *N*,*N*-dimethylformamide (1400 ml) and 1,6-dimethylpyrimido[5,4-*e*]-1,2,4-triazine-5,7(1*H*,6*H*)-dione (**2**, 112 g, 0.58 mole) was heated at reflux for 4.5 hours and then concentrated to a tarry, dark brown residue. This material was dissolved in methanol (1500 ml), Celite (500 g) was added, and the methanol was removed *in vacuo*. The residual powder was extracted in a Soxhlet apparatus with ether (10 l) until the ether layer was nearly colorless (*ca.* 10 days). Concentration of the ether gave crude **1** (*ca.* 50 g), which was purified *via* filtration chromatography on silica gel employing 4% methanol-chloroform as eluent. Thus was obtained pure **1** (39.3 g, 38%) as a bright yellow powder, mp 239.5-240.5° (lit [6], 239-240°); ir (potassium bromide): 3414 (NH), 1737 (C=O), 1684 (C=O) cm^{-1}; nmr (DMSO-d_6): 12.67 (br s, 1H, N*H*), 9.71 (s, 1H, Ar*H*), 3.27 (s, 3H, CH_3); tlc (silica gel; methanol:chloroform, 1:9) R_f 0.45.

Anal. Calcd. for $C_6H_5N_5O_2$ (179.14): C, 40.22; H, 2.81; N, 39.10. Found: C, 40.53; H, 2.92; N, 38.94.

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REFERENCES AND NOTES

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[13] This procedure is necessary to avoid spontaneous ignition of the solvent.

[14] Xanthothricin (toxoflavin) is **extremely** toxic and should be handled with great care.