A New Antibacterial Agent (U-20,661) Isolated from a Streptomycete Strain

Antibiotic U-20,661 is synthesized in the culture broth by a newly isolated *Streptomyces* strain designated *Streptomyces steffisburgensis* to be described by DIETZ[1].

Fermentations were conducted under submerged culture conditions in shaken flasks in a medium containing corn steep liquor, 20 g/l; glycerol, 20 g/l; NaCl, 5 g/l; tap water to 1 l; pH was adjusted to 7.2 with NaOH before sterilization. Elaboration of the antibiotic was measured by a microbiological disc-plate assay[2] with *Sarcina lutea* as the test organism. The log dose-response curve was linear over a concentration range of 10–50 μg/ml. Peak titers were usually obtained after 5 days of incubation at 28 °C.

The antibiotic was isolated from a 4 l aliquot of filtered fermentation broth. The broth was extracted at pH 6.0 with 3.6 l methylene chloride. The extract was concentrated in vacuo at < 40 °C to dryness, and the residue was dissolved in 250 ml of boiling isopropanol. The solution was again clarified by filtration, concentrated in vacuo to a volume of 65 ml, and heated to 50 °C. Crystallization started at this temperature and the mixture was held overnight at 0 °C. The crystals were removed by filtration, washed with fresh isopropanol and dried in vacuo at room temperature to a constant weight of 450 mg.

Crystalline antibiotic U-20,661 is an orange-yellow compound. Acidic aqueous solutions exhibit a bright yellow color and will turn to an intensive purple upon addition of strong base. The UV- and visible spectra, determined in methanol, 0.01 *N* methanolic HCl, and 0.01 *N* methanolic KOH, respectively, show absorption at the following wavelengths (Table I).

The IR-spectrum in mineral oil suspension (Figure 1) shows absorption at the following frequencies: 3470, 2940, 2920, 2850, 2720, 1710, 1672, 1620, 1610, 1605, 1560, 1510, 1463, 1445, 1410, 1395, 1376, 1310, 1293, 1268, 1260, 1245, 1228, 1212, 1193, 1163, 1143, 1123, 1105, 1070, 1053, 1037, 1020, 1005, 970, 962, 925, 913, 893, 870, 858, 832, 812, 800, 793' 769, 745, 725, 698, 690 cm^{-1}.

The antibiotic has a specific rotation ($[\alpha]_D^{25}$) of +85° (c. 0.05% in methanol) and melts at 257-265 °C (hot stage). Its solubility is greater than 5 mg/ml in methanol, chloroform, acetone, dimethylformamide, 1-butanol, methyl ethyl ketone, ethyl acetate and less than 5 mg/ml in water, diethyl ether, and cyclohexane.

Table I. UV- and visible absorption spectra of antibiotic U-20,661

Methanol	Maximum at	214 nm, log ε = 4.41
	Maximum at	236 nm, log ε = 4.47
	Inflection at	255 nm, log ε = 4.35
	Maximum at	278 nm, log ε = 4.30
	Inflection at	298 nm, log ε = 4.13
	Maximum at	439 nm, log ε = 4.17
0.01 *N* methanolic HCl	Maximum at	214 nm, log ε = 4.40
	Maximum at	236 nm, log ε = 4.47
	Inflection at	255 nm, log ε = 4.35
	Maximum at	278 nm, log ε = 4.30
	Inflection at	300 nm, log ε = 4.11
	Maximum at	439 nm, log ε = 4.18
0.01 *N* methanolic KOH	Maximum at	227 nm, log ε = 4.47
	Maximum at	263 nm, log ε = 4.39
	Maximum at	353 nm, log ε = 3.62
	Maximum at	528 nm, log ε = 4.05

The paper chromatography pattern in 9 solvent systems is shown in Figure 2. The molecular weight was found to be 568 ± 8 by X-ray crystallography and 574 by mass spectrometry.

Table II. In vitro antibacterial activity of antibiotic U-20,661 in brain-heart infusion broth

Test organism	Minimal inhibitory concentration, μg/ml
E. coli ATCC 26	> 1000
K. pneumonias PCI 602	> 1000
P. vulgaris ATCC 8427	> 1000
Ps. aeruginosa ATCC 9027	> 1000
S. paratyphi U 263	> 1000
S. pullorum MSDH 75	> 1000
S. typhi MSDH TG3	> 1000
S. aureus UC 76	31
S. aureus UC 70	31
S. faecalis ATCC 6057	31
S. hemolyticus C 203	31
S. viridans UC 155	31

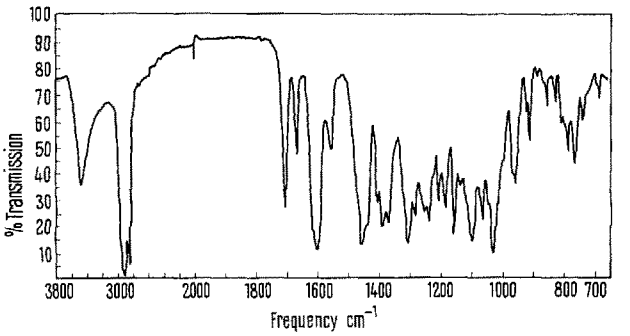

Fig. 1. IR-absorption spectrum of antibiotic U-20661 (in mineral oil suspension).

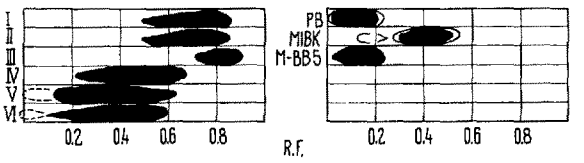

Fig. 2. Paper chromatographic patterns of antibiotic U-20661. Solvent systems: (I) 1-butanol-water (84:16) developed 16 h; (II) 1-butanol-water (84:16) plus 0.25% *p*-toluenesulfonic acid, developed 16 h; (III) 1-butanol-acetic acid-water (2:1:1), developed 16 h; (IV) 1-butanol-water (84:16) plus 2% piperidine, developed 16 h; (V) 1-butanol-water (4:96), developed 5 h; (VI) 1-butanol-water (4:96), plus 0.25% *p*-toluenesulfonic acid, developed 5 h; (PB) 0.1 *M* KPO_4 buffer, pH 7.0, developed 5 h; (MIBK) 0.075 *N* NH_4OH saturated with methyl isobutyl ketone, developed 5 h; (M-BB5) benzene-methanol-water (1:1:2), paper strip was equilibrated at 25 °C in vapor from the mixed solvent and developed for 5 h with upper phase. The antibiotic was located by bioautography on agar trays seeded with *Sarcina lutea*.

[1] A. DIETZ, in preparation.

[2] L. J. HANKA, D. J. MASON and W. T. SOKOLSKI, *Antibiotics Chemother. 11*, 123 (1961).

Analysis calculated for $C_{28}H_{30}O_{13}$ (M.W. 574.5) was C, 58.53; H, 5.26; O, 36.21. Found: C, 58.31; H, 5.52; O, 35.89.

Antibiotic U-20,661 inhibits gram-positive bacteria in vitro (Table II) but is inactive against gram-negative bacteria when assayed in a two-fold broth dilution test[3]. No antibacterial activity was observed in mice experimentally infected with *Streptococcus hemolyticus*[3] when treated s.c. at the maximum tolerated dose. No blood serum levels were demonstrated at the same dose and administration route. The antibiotic was inactive against T_6-phage in *E. coli* assayed according to ADAMS[4].

Antibiotic U-20,661 inhibits the growth of KB-cells yielding an ID_{50} (50% inhibition of protein synthesis) of 1.6 μg/ml using the assay system described by SMITH et al.[5]. The antibiotic is, therefore, extremely cytotoxic against mammalian cells grown in vitro. Conversely, the compound is remarkably non-toxic in mice. Maximum tolerated doses were 800 mg/kg day orally, 400 mg/kg day s.c. or 100 mg/kg day when administered i.p. The acute LD_{50} (i.p.) was 562 mg/kg in mice.

Zusammenfassung. Ein neues Antibiotikum, U-20661, wurde aus der Kulturflüssigkeit eines Streptomycetenstammes isoliert, welches in vitro nur gegen grampositive Bakterien wirksam ist. Die Substanz ist extrem cytotoxisch in Gewebekulturen, aber ungiftig für Mäuse.

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[3] C. LEWIS, H. W. CLAPP and J. E. GRADY, *Antimicrobial Agents and Chemotherapy* (Published by the American Society for Microbiology, 1962), p. 570.
[4] M. H. ADAMS, *Bacteriophages* (Interscience Publishers Inc., New York 1959), p. 450.
[5] C. G. SMITH, W. L. LUMMIS and J. E. GRADY, *Cancer Res. 19*, 847 (1959).

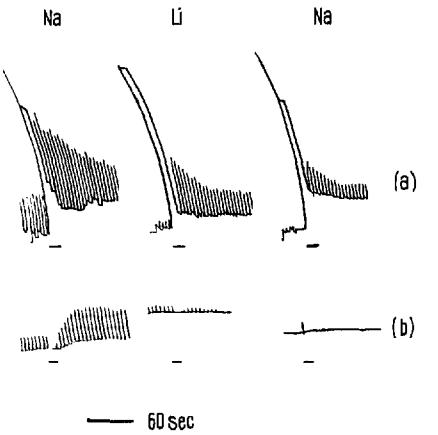
The Effect of Lithium Ions on the Post-Tetanic Potentiation of Neuro-Muscular Transmission

In partially curarized nerve-muscle preparations, neuro-muscular transmission recovers after a period of repetitive activity[1]. This recovery seems to be due to increased transmitter liberation following tetanus[1,2]. It has been suggested that the increase is caused by changes in action potential associated with the post-tetanic hyperpolarization[3]. Since the post-tetanic hyperpolarization is greatly reduced or abolished when the extracellular Na is replaced by Li[4,5] the effect of Li on neuromuscular transmission and on post-tetanic potentiation was studied.

Isotonic contractions and nerve action potentials were recorded from the guinea-pig hemidiaphragm-phrenic nerve preparations[6] suspended in Krebs, or modified Krebs solution, to which $3 \cdot 10^{-4} M\ L^{-1}$ choline had been added. The solutions were equilibrated with 95% O_2/5% CO_2 and kept at 35–37 °C. The nerve was stimulated so that maximal muscle twitches were obtained and D-tubocurarine or Mg was added until the muscle twitch was reduced to about $^1/_5$ of its maximal amplitude. Stimulation was maintained at a rate of 0.2 shocks/sec and was then increased for 10 sec to 200 shocks/sec. After the period of tetanic stimulation, the muscle contractions were several times larger than before the tetanus. Further stimulation at 0.2 shocks/sec often gave a few contractions of increasing amplitude. The amplitude of the subsequent contractions gradually returned to the pre-tetanic level within 20–60 sec (Figure). In preparations blocked with D-tubocurarine, the muscle did not contract during the tetanus; in Mg-blocked preparations tetanic muscle contractions were observed. Direct stimulation of the muscle in these preparations showed that the muscle twitches were not increased after the tetanus.

When the Krebs solution was replaced by a solution in which Li had been substituted for Na, neuro-muscular transmission in non-curarized preparations was maintained for about 8 min. The muscle twitches then decreased and neuro-muscular block occurred after 15 min.

Post-tetanic potentiation was found in curarized preparations kept in Li-solution. The potentiation was about the same as in Na-solution when the preparations



Effect of Li on post-tetanic potentiation in partially curarized hemidiaphragm-phrenic nerve preparation. Nerves were stimulated at 0.2/sec and stimulation frequency increased to 200/sec for 10 sec (—). Left-hand records were taken with preparations in Na, centre records in Li, and right-hand records again in Na solution. In experiment (a) solutions contained $1.1 \cdot 10^{-5} M$ Mg and pre-tetanic exposure to Li was 5 min; in experiment (b) the preparation was curarized with $1.5 \cdot 10^{-9} M$ D-tubocurarine and exposure to Li was 10 min at 37 °C.

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