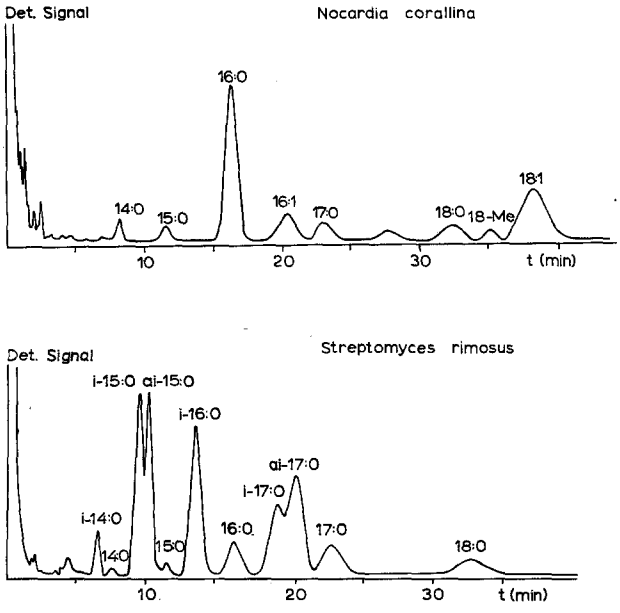
ployed, as well as the characterization of all actinomycetes of our collection which have been investigated, will be presented elsewhere.

Results and discussion. The results obtained with 62 strains of 41 species and 16 genera of *Actinomycetales* are listed in the Table. For the purpose of classification, it suffices to indicate the presence or absence of a certain class of fatty acids. Further, only fatty acids with more than 2% of total acids were considered (for quantitative data of the individual acids, see the forthcoming publication). The DAP separates the *Streptomycetaceae* (Table, No. 5, 6 and 7) with LL-DAP from all other *Actinomycetales*, most of which contain DL-DAP; in *Actinomyces* the DAP of the peptidoglycan is replaced by lysine. Numerous actinomycetes with DL-DAP can be differentiated by their sugar and or fatty acid spectrum. However, *Mycobacterium* and the *Nocardiaceae* (No. 3 and 4) exhibit both the sugar type A with arabinose and galactose and the same fatty acid spectrum. These organisms can be differentiated by their mycolic acid type[10,11]. As already shown in the early studies[3,4] the occurrence of xylose and madurose (sugar type D and B respectively) are of diagnostic value. Here we want to add to these well-known features the fatty acid spectrum. If only the 4 classes of acids 1. saturated, 2. iso- and anteisobranched, 3. 10-methyl and 4. unsaturated, are taken into consideration, the following patterns emerge: 1. +−−+: *Actinomyces*, 2. +−++: *Mycobacterium, Nocardiaceae*, 3. ++−−: *Streptomycetaceae, Actinoplanes, Amorphosporangium, Thermoactinomyces, Saccharomonospora*, 4. +++−: *Planobispora, Streptosporangium, Micromonospora, Microbispora*, and 5. ++++: *Actinomadura*. The distribution of these patterns shows that variation exists in some of the present families: Actinoplanaceae and Thermomonosporaceae. The 3 biochemical markers discussed in this paper, as well as the distribution of mycolic acids of various types among *Actinomycetales*[10,11], will be of great help to the actinomycete taxonomist.



Gaschromatographic resolution of fatty acid esters from *Nocardia corallina* and *Streptomyces rimosus*. (Parameters: Inj. port: 250 °C; oven: 155 °C; detector 300 °C; carrier gas N_2: 30 ml/min; hydrogen: 30 ml/min; air 240 ml/min; sample 1 µl.)

[10] M. P. LECHEVALIER, A. C. HORAN and H. A. LECHEVALIER, J. Bact. *105*, 313 (1971).

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Emericid*, a New Polyether Antibiotic from *Streptomyces hygroscopicus* (DS 24 367)

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Summary. Emericid is a new polyether polycyclic ionophore antibiotic excreted by *Streptomyces hygroscopicus* (DS 24 367). Active in vitro against Gram-positive bacteria, it is ineffective in vivo. At a 0.006–0.02% level in the diet it protects chickens and rabbits against coccidiosis.

Recent research on the production of antimicrobial agents by microorganisms have led to the discovery of emericid (31 559 R.P.), a new antibiotic isolated from the culture broths of *Streptomyces hygroscopicus* DS 24 367.

The strain, which was isolated from a sample of soil collected abroad and selected by classical tests of antibiosis[1], exhibits all the main morphological and biochemical features of the species *Streptomyces hygroscopicus*, as described by several authors[2,3]. It is stored as a dry spores and sterile soil mixture.

Emericid is produced by submerged culture as follows: the strain is first grown from the spores on BENNETT's agar medium[4] in test tubes for 15 days at 26 °C. A suitable inoculum is obtained by successive transfers of the tube culture; first into 250 ml of a liquid medium (composition in g/l: yeast extract 15, anhydrous glucose 10, agar 2) in a 2 l flask incubated for 48 h at 26 °C on a rotary shaker, then into 40 l of another medium (in g/l: peptone 10, yeast extract 5, glucose monohydrate 10, agar 2, pH before sterilization 7,3) contained in a stirred and aerated 75 l fermenter. After 25 h at 27 °C this last

* From a recent paper by N. OTAKE (Tetrahedron Lett. *1970*, 4147) emericid and lonomycin would be identical.

[1] S. A. WAKSMAN, *Microbial Antagonisms and Antibiotic Substances* (The Commonnwealth Fund, New York 1954).

[2] H. D. TRESNER and E. J. BACKUS, Appl. Microbiol. *4*, 243 (1956).

[3] S. A. WAKSMAN, *The Actinomyces II* (The Williams and Wilkins Company, Baltimore 1961), p. 230.

[4] See[3], p. 331.

culture is poured as a whole into a 800 l fermenter containing 400 l of the following production medium (in g/l): dried kidney beans 25, distillers' solubles 5, glucose monohydrate (separately sterilized) 10, glycerine 15, sodium chloride 3, hydrated cobalt chloride 0,02, pH before sterilization being 7,5. The culture is aerated, stirred and kept at 27 °C. The production of emericid reaches 500 µg/ml at 120 h.

Practically the whole antibiotic is retained by the mycelium. The cell mass, collected by filtration at pH 3 of the broth including Clarcel DIC (CD) as an adjuvant, is dispersed into 300 l of methanol at pH 10. After filtration, the organic solution is reduced to 4% of its initial volume by distillation under low pressure. By this way, a crude product containing 80% of the sodium salt of emericid is precipitated.

Sodium salt of pure emericid is obtained by dissolving the crude product in acetone, dimethylformamide or methanol and diluting with water. It is a fine colourless crystalline solid, m.p. 220°C with decomposition (KOFFLER); $[\alpha]_D^{20} = +51,2° \pm 1,0°$ (c = 1 in methanol). It is practically insoluble in water, sparingly soluble in *n*-hexane, soluble in alcohols, ketones, dimethylformamide and ethylacetate, easily soluble in chlorinated solvents. There is no UV-absorption peak as far as 210 nm. The IR-spectrum (KBr pellet) is given in the Figure 1.

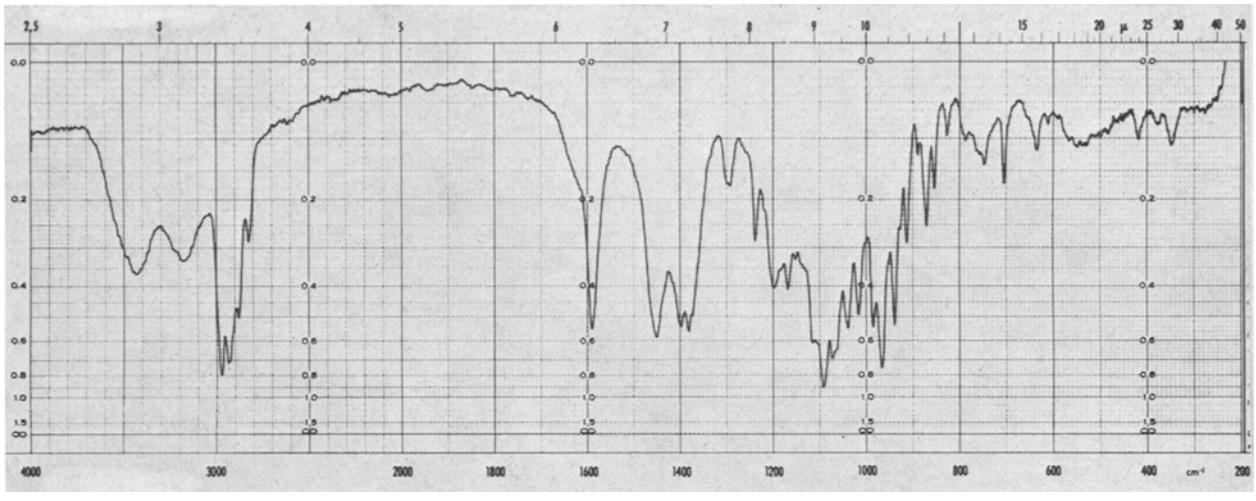

Fig. 1. IR-absorption-spectrum of emericid (KBr pellet).

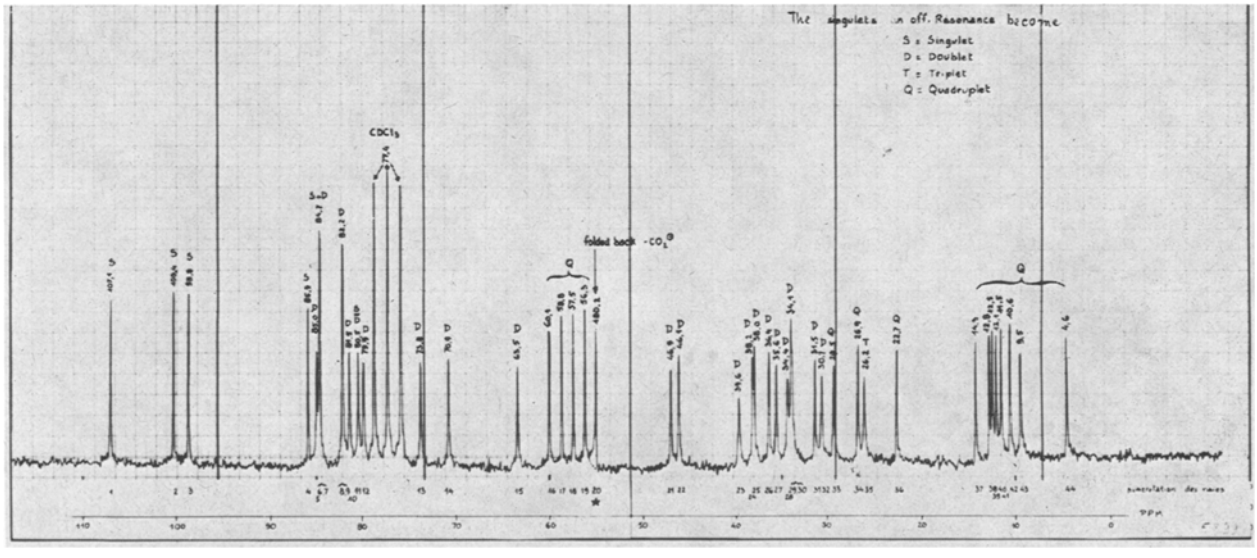

Fig. 2. ^{13}C NMR-spectrum of emericid.

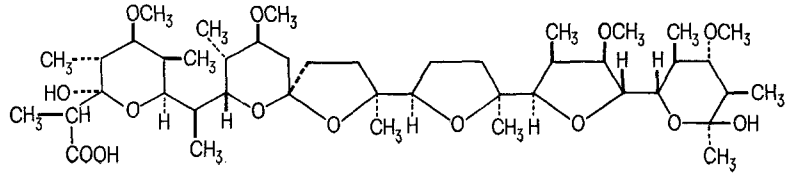

Fig. 3. Planar structure of emericid.

Bacteriostatic activity of Emericid

Test organism	Minimum inhibitory concentration (μg/ml)
Staphylococcus aureus (strain 209 P, ATCC 6 538 P)	0.8
Staphylococcus aureus (strain Smith)	2.5
Sarcina lutea (ATCC 9341)	1.9
Streptococcus faecalis (ATCC 8043)	0.5
Streptococcus pyogenes hemolyticus (strain Dig 7, Institut Pasteur)	0.8
Diplococcus pneumoniae (strain Til, Institut Pasteur)	0.2
Neisseria catarrhalis (A 152, Institut Pasteur)	50
Bacillus subtilis (ATCC 6633)	0.8
Bacillus cereus (ATCC 6630)	0.5
Mycobacterium species (ATCC 607)	25
Escherichia coli (ATCC 9637)	> 150
Shigella dysenteriae (Shiga L, Institut Pasteur)	> 150
Salmonella paratyphi A (strain Lacasse, Institut Pasteur	> 150
Salmonella schottmuelleri (paratyphi B; strain Fougenc, Institut Pasteur)	> 150
Proteus vulgaris	> 150
Pseudomonas aeruginosa (strain Bass, Institut Pasteur	> 150

Elemental composition: C 63.2%, H 8.8%, O 24.3%, Na 2.9% is in agreement with the hypothetic molecular formula $C_{44}H_{75}O_{14}Na$; m.W.: 851.06; neutral equivalent with perchloric acid in acetic acid: 840.

According to its physicochemical properties, mainly the peculiar solubility of its sodium salt, emericid seems to belong to the family of ionophore cyclic polyethers[5]. As a matter of fact, proton and 13 C NMR-spectrometry (Figure 2) has shown that emericid owns the same chain of cyclic polyethers as nigericin[6] and grisorixin[7] with only minor differences for the side-substituents. Moreover, attempts to identify emericid with some other products of the family, especially alborixine[8], dianemycin[9], lasalocid[10], lysocellin[11], monensin[12], A 204[13], A 28 695 A and B[14], by thin layer chromatography have been unsuccessful (conditions: spot 100 μg in methylene chloride on a Silicagel Merck F 254 plate; develop with the light phasis of a cyclohexane, ethylacetate, water and butanol 50-50-25-5 mixture; spray with a vanillin 3 g, methanol 100 ml and concentrated sulfuric acid 0.5 ml reagent and heat at 105 °C). Finally, the uniqueness of this compound was substantiated by means of X-ray -crystallography of the silver salt, unraveling its structural formula as shown by Figure 3[15].

The LD_{50} of emericid is 150 mg/kg for the chicken, with a single p.o. administration.

The bacteriostatic activity of emericid against some microorganisms is shown in the Table. The minimum inhibitory concentration determinations were carried out by the dilution method in appropriate medium for each organism and after incubation for 18 h at 37 °C. Emericid is only active in vitro against gram-positive bacteria and practically inactive against any other type of bacteria, yeasts and fungi. In vivo, emericid is inactive against staphylococcal and pneumococcal infections of mice by the oral or s.c. route.

As previously shown[16], emericid is an excellent coccidiostat in chickens; mixed with the feed at 0.006–0.02% levels according to the infecting species of Eimeria, it suppresses mortality and intestinal lesions, reduces oocysts excretion and allows a normal growth of the infected birds; there is no emergence of resistant Eimeria strain during the treatment. Similar results have been obtained in rabbits.

In conclusion, emericid is a new ionophore polycyclic polyether, active in vitro against gram-positive bacteria but devoid of any in vivo antibacterial activity. Its main interest lies in the eradication of coccidiosis in chickens and rabbits at the 0.006–0.02% level in the diet.

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Effect of Cyclic AMP and Theophylline on Phagocytotic Activity of *Tetrahymena pyriformis*

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Summary. Dibutyril cAMP and the cPDE-inhibitor theophylline both enhance the phagocytotic activity of *Tetrahymena*. Theophylline and cAMP-activating histamine are synergistic. It follows that the cAMP-adenylcyclase system functions in the unicellular animal *Tetrahymena*.

Certain forms of hormonal regulation have been shown to be operative in unicellular organisms, although they are seemingly of little consequence at this stage of phylogenesis. Epinephrine[1,2] and serotonin[3] have been shown to be present in *Tetrahymena* at changing concentrations, depending on the functional stage of the protozoon. Also, *Tetrahymena* was found to respond to certain hormones not present in it and not encountered by it under natural conditions. Histamine[4] produced a considerable increase in the phagocytotic activity of the protozoon, whereas

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