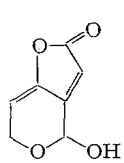
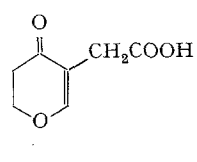
Desoxypatulinic Acid from a Patulin-Producing Strain of *Penicillium patulum*

Desoxypatulinic acid, previously obtained by synthesis[1] and by hydrogenation of patulin[2], has not been reported as a natural product. We have now isolated it from cultures of *Penicillium patulum* Bainier (*P. urticae* Bainier), a known source of many fungal metabolites, including patulin[3,4].

P. patulum[5], strain No. 562 isolated from a mixed feed suspected of causing death of a number of cattle, was grown at room temperature in 2.8-liter Fernbach flasks each containing 200 ml of yeast extract (2%)-sucrose (15%) liquid medium. After 9–11 days incubation, the medium was extracted with 3 equal volumes of ethyl acetate. The solvent was evaporated, and the residue dissolved in warm benzene and chromatographed on a column of silica gel. Elution with benzene-ethyl acetate (9:1, v/v) yielded patulin (I) (124 mg per Fernbach flask), m.p. 110–111 °C after recrystallization from benzene; it was identified by comparison with standard material (mixed m.p., UV- and IR-spectra, and thin-layer chromatography (TLC)). Further elution of the column with benzene-ethyl acetate (85:15, v/v) gave fractions containing griseofulvin (identified by comparative TLC). A more polar compound (80 mg crystalline material per Fernbach flask) was eluted by benzene-ethyl acetate (85:15 to 75:25, v/v). It was purified by crystallization from *iso*-propyl ether-*n*-hexane and toluene followed by sublimation. The crystals, m.p. 115–115.5 °C, were soluble in water (to give an acidic pH) and aqueous $NaHCO_3$ (with effervescence). The molecular formula was $C_7H_8O_4$ (found: C, 53.94; H, 5.16. Calc. for $C_7H_8O_4$: C, 53.85; H, 5.16%). UV- and IR-spectral properties were λ_{max} (EtOH) 267 nm (ε 7960); ν_{max} ($CHCl_3$) 3510 (OH), bands between 3000 and 2400 (COOH), 1718 (COOH dimer), 1672 (conjugated C=O), 1621 (C=C) cm^{-1}. The 100 MHz NMR-spectrum (in $CDCl_3$) consisted of signals at τ 1.53 (singlet, 1P, disappears on addition of D_2O; OH), 2.63 (singlet, 1P; CO.C=CH.O), 5.48 (triplet, 2P, J=7Hz; CH_2O), 6.83 (singlet, 2P; CO · CH_2 · C = C), and 7.34 (triplet, 2P, J = 7Hz; CO · CH_2); each triplet collapsed to a singlet on irradiation at the frequency of the other triplet. The mass spectrum (70 e.v.) showed a parent ion at *m/e* 156 and other prominent ions at *m/e* 112 ($M-CO_2$), 83, 60 [$CH_2 = C(OH)_2^+$], 55, 39 and 27[6]. Consideration of the foregoing evidence led to formulation of the metabolite as 2, 3-dihydro-4-pyrone-5-acetic acid (II), desoxypatulinic acid [m.p. 114.5–115.5 °C; λ_{max} (EtOH) 268 nm][1,2]. This assignment was confirmed by direct comparison of the metabolite (mixed m.p., TLC, and IR-, UV- and mass spectra) with desoxypatulinic acid, m.p. 113.5–114.5 °C, prepared by hydrogenolysis of chlorodesoxypatulinic acid[2]. Desoxypatulinic acid (46 µg/ml tryptic soy agar) had no inhibitory effect on *Bacillus megaterium, B. subtilis, B. cereus, Staphylococcus aureus, S. epidermidis, Sarcina lutea, Micrococcus flavus*, and *Saccharomyces cerevisiae* (cf. patulin was completely inhibitory under the same conditions).



I



II

Desoxypatulinic acid was readily detected by TLC on silica gel F-254 (0.25 mm) developed with toluene-ethyl acetate-formic acid (6:3:1, v/v) as a dark spot under short wavelength UV-light at Rf 0.22. Analysis by TLC of aliquots of culture medium withdrawn during the fermentation showed that desoxypatulinic acid and patulin (Rf 0.33) concentrations reached a peak at about the same time (10–12 days); patulin was no longer detectable on day 17 although traces of the acid remained. The relationship of desoxypatulinic acid to the pathway of patulin biosynthesis[4,7,8] merits investigation.

Zusammenfassung. Aus Kulturlösungen von *Penicillium patulum* Bainier wurde neben Patulin die Desoxypatulinsäure isoliert und identifiziert.

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5 The fungus was identified at the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.
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7 S. W. TANENBAUM and E. W. BASSETT, J. biol. Chem. *234*, 1861 (1959).
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Systematics of a Leptospira Strain Isolated from Frog

In the year 1964, in Iowa, USA, DIESCH et al.[1,2] isolated a leptospira strain from a pool of kidneys of 3 specimen of frogs (*Rana pipiens*) collected during follow-up of a human leptospirosis outbreak associated with swimming.

This is the first case reported in the literature of isolation of a leptospira strain from the organs of an amphibian. Therefore, it is of particular interest, also for epidemiological purposes, to ascertain whether this strain belongs to pathogenic leptospiras (*L. interrogans*) or to saprophytic ones ('biflexa' complex), and whether this strain might be inserted in the group of leptospiral serotypes that we know already. DIESCH et al.[3] have executed some surveys in this direction without being able to attain a sure conclusion. In fact, the leptospira, inoculated into guinea-

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pigs, hamsters, gerbils and mice, did not cause in these animals any pathologic manifestation and was not isolated from their blood or from their organs. When inoculated into frogs, the leptospira only caused a slight and inconstant antibody response. It could not be recovered from the organs of these amphibians.

Serological tests were executed, by the agglutination test, comparing the strain isolated to leptospiras belonging to 13 pathogenic serotypes and to a strain of the 'biflexa' complex. It was not possible to ascertain any consistent antigenic affinity between the strain of the frog and the leptospira strains compared to it.

It is interesting to point out that the serum of the frogs constituting the pool from which the leptospira has been isolated did not contain any agglutinins for this strain.

We have received the strain in question, marked I.C.F. from Dr. DIESCH, whom we warmly thank for his kindness. The strain has been maintained here in Korthof-Babudieri's medium.

Firstly, we have submitted the strain to the biochemical tests which permit us to differentiate pathogenic leptospiras from saprophytic ones. More precisely, the strain has been inseminated in medium containing 8-azoguanine (420µ/ml) copper sulphate (1:100,000) and peptone plus bicarbonate, according to MAZZONELLI[4]. The leptospira has not developed in any of these media, this behaving like a typical pathogenic leptospira.

The leptospira has been inoculated i.p. into young guinea-pigs. They did not show any sign of disease. It was recovered from the blood 1 h after inoculation; not later. It could not be recovered from the liver or kidney of the animals sacrificed after 7 days.

Later on, after preparing with it an immune serum at a high titer (1:1,000,000), the leptospira under study was compared, by cross-agglutination test, to the reference strains of all the serotypes of pathogenic leptospiras known so far[5], as well as to a leptospira strain recently isolated in the Philippines Islands, from the kidneys of a toad (strain 3-C) and still under study. The strains employed in this test were, altogether, 141. None of these strains was found to have a significant antigenic affinity with the strain I.C.F. It only showed a very slight affinity with the *javanica* serotype. Consequently, we can affirm that the strain I.C.F. belongs to the group of pathogenic leptospiras and that it represents in this group a new serogroup and a new serotype, for which we suggest the name '*ranarum*'.

It is interesting to realize that amphibians may be carriers of pathogenic leptospiras. However, in the case we are examining, the scarce virulence shown by the strain I.C.F. for common laboratory animals and even for frogs, and the fact that it belongs to a new serotype, which so far has never been acknowledged to be responsible for cases of leptospirosis in human beings or in domestic animals, makes us presume that the epidemiological importance of this leptospira is very limited.

Zusammenfassung. Nachweis, dass Nieren von *Rana pipiens* Träger pathogener Leptospiren sein können und dass in deren System mit dem neuen Leptospirenstamm eine bisher unbekannte Serogruppe aufgefunden wurde, für welche der Name '*ranarum*' vorgeschlagen wird.

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THEORIA

On the Molecular Mechanism of Action of the Tetracyclines

The tetracyclines are known to exert their antibiotic activity by binding to the 30 S ribosome and blocking the binding of aminoacyl t-RNA[1]. Since the ribosomes are constructed of RNA and protein it may be possible to suggest the nature of the binding site of tetracyclines by a comparison of molecular models of the drug with possible variations of RNA, protein or RNA-protein structures available to bind the small, complex and fixed molecule of a typical tetracycline.

Material and methods. As described in a previous communication[2] Corey-Pauling-Kaltun models of a variety of tetracyclines were prepared as well as segments of various RNA and protein structures and their relationships examined.

Results. The –OH, =O, –OH grouping common to all tetracyclines is complimentary to the NH, NH, O grouping of an Arg-Glu ionically bonded pair or to the N:, NH, O grouping of G-C (minor side) and A-U (major side) base pairs. Therefore, as working hypotheses we set up 1. two protein segments (β-pleated sheet conformation), joined by 2 Arg-Glu ionic (double resonating) cross links, and 2. a segment of RNA with various base pairs. We could observe no particular relationship between the tetracycline molecule and structure 1, but a close relationship became apparent to a fully extended segment of RNA (double strand) of sequences GC:GC or GC:AU as shown in Figures 1 and 2. The tetracycline molecule sits on the minor groove side and forms a roof over the gap between the separated base pairs. It binds as follows (hydrogen bonds except where stated: the numbers refer to the bonds marked in Figure 1): **1**, 10 OH group to cytosine 0; **2**, 11 = 0 from guanine NH; **3**, 10 OH to guanine 3*N*; **4**, the OH between C_{12} and C_1 to ribose ring 0; **8**, the basic NH^+ to phosphate O^- (ionic); **7**, the 3OH to the G (or A) 3*N*: of the second pair; **5**, the 2 C=O from the ribose 2OH of the second pair purine base (weak interaction); **9**, the 6OH to C=O (or U=O) of the second pair and (**10**, **11**, **12**) the C7, 8, 9 hydrogen (and 7 Cl of chlortetracycline and demeclocyline) make lipophilic contacts with ribose 1 and 4 CHs of the second pair and the ribose 4CH of the first pair, respectively. The NH_2 group **6**, (or NH.CH_2.N (C_4H_8) group of rolitetracycline) intercalates between the purine π clouds. There is nothing to indicate whether the purine of pair 2 is guanine or adenine since the 5 position

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