Communications to the editor

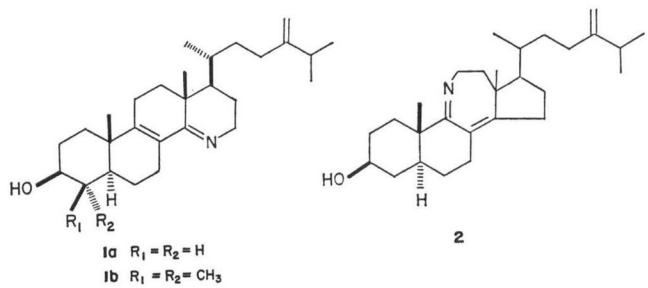
STRUCTURE OF ANTIBIOTIC A 25822 B, A NOVEL NITROGEN-CONTAINING C_{28}-STEROL WITH ANTIFUNGAL PROPERTIES

Sir:

The mold *Geotrichum flavo-brunneum* was found to produce a complex of closely related compounds with broad-spectrum antifungal activity (K. MICHEL *et al.*, to be published).

The principal active component, A 25822 B (m.p. 115~118°C, $[\alpha]_D^{25}$ −20° [*c* 0.775, MeOH], pKa′ 8.4 [66% DMF], λ_{max} [EtOH] 238 nm [ε 12,300], γ_{max} [$CHCl_3$] 3571, 1618 cm^{-1}), has the molecular formula $C_{28}H_{45}NO$ ($M^{+\cdot}$ 411.3497; required, 411.3501). A shift of the maximum to 277 nm (ε 13,400) in acidic solution suggested that u.v. absorption was due to the presence of a conjugated imine group; *i.e.*, C=C−C=N[1~3]. This was supported by the observation that A 25822 B reacted with KBH_4 in CH_3OH[2,4] to give an amorphous dihydro derivative ($M^{+\cdot}$ 413; no u.v. λ_{max} at 238 nm). The latter formed a crystalline diacetate (m.p. 130~132°C; $M^{+\cdot}$ 497.3869; required for $C_{32}H_{51}NO_3$, 497.3869; γ_{max} [$CHCl_3$] 1706 [ester C=O] 1639 [amide C=O] cm^{-1}). The nmr spectrum (Fig. 1) revealed the presence of several methyl groups and exhibited a broad envelope of absorption due to methine and methylene protons. Two olefinic protons (δ 4.67 [$J<1.0$], 4.75 [$J<1.0$]) were assigned to an exomethylene group because of the characteristic small, vinyl geminal coupling (<1.0 Hz).

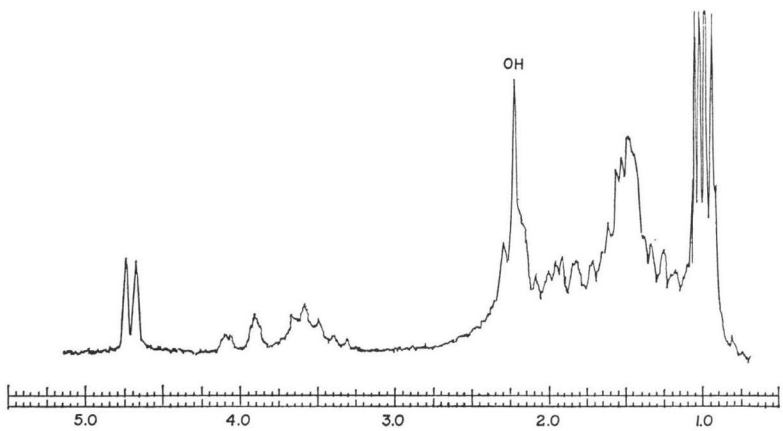
On the basis of the foregoing information, together with additional spectroscopic and chemical data that will be reported at a later time, two possible structures, **1a** and **2**, were considered for A 25822 B. Conclusive evidence for structure **1a** was obtained by X-ray crystallographic analysis of its methiodide derivative. The latter crystallized from CH_3OH-H_2O solu-



1a $R_1 = R_2 = H$
1b $R_1 = R_2 = CH_3$

2

Fig. 1. NMR spectrum of A25822B (100 MHz, $CDCl_3$)



tion as yellow plates. The crystals belong to the noncentrosymmetric space group $P2_12_12_1$, with four molecules in a unit cell having the dimensions $a=8.248\pm0.002$ Å, $b=7.804\pm0.002$ and $c=46.33\pm0.02$. The crystal density measured by flotation is 1.230 g cm^{-3}, compared to a calculated density of 1.233 g cm^{-3} for $C_{28}H_{45}NO\cdot CH_3I$. Intensities for 1454 unique reflections were measured on an automated diffractometer using copper X-radiation. The position of the iodide ion was determined from a sharpened PATTERSON map. Calculation of a three-dimensional electron density map phased on the iodide revealed all of the non-hydrogen atoms except six on the end of the side chain. The structure was partially refined by leastsquares and a difference map calculated to find the remaining non-hydrogen atoms. Further refinement brought the R value down to 0.16. Complete details of the refined crystal structure will be published elsewhere.

A related minor factor, A 25822 A (m. p. 147°C, $[\alpha]_D^{25}$ −72° [*c* 1.15, MeOH], pKa′ 8.0 [66% DMF], λ_{max} 239 nm [ε 12,600], γ_{max} 3584 [OH], 1621 [C=C−C=N] cm^{-1}), was shown to be the 4,4-dimethyl analog of A 25822 B (**1b**). It has the molecular formula $C_{30}H_{49}NO$ ($M^{+\cdot}$ 439.3776; required, 439.3814).

Compounds **1a** and **1b** are thus members of a new family of mold metabolites. To the best of our knowledge, they are the first reported examples of naturally-occurring homo-aza sterols. Details of their isolation and properties and, also, other factors of the complex will be reported in subsequent papers from these laboratories.

Acknowledgement

The authors thank Mr. K. MICHEL and Mr. S. LARSEN for the antibiotics used in this investigation.

J. W. CHAMBERLIN
M. O. CHANEY
S. CHEN
P. V. DEMARCO
N. D. JONES
J. L. OCCOLOWITZ

The Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, Indiana 46206, U.S.A.

(Received September 2, 1974)

References

1) SCOTT, A. I.: Interpretation of the Ultraviolet Spectra of Natural Products. p. 81, Pergamon Press, Oxford, 1964
2) GUREVICH, A. I.; M. N. KOLOSOV, V. G. KOROBKO & V. V. ONOPRIENKO: The structure of abikoviromycin. Tetrahedron Letters 1968: 2209~2212, 1968
3) WONG, J. L. & M. H. RITCHIE: α- and β-Pyrrolenines *via* a pyrrole GRIGNARD reaction. Chem. Commun. 1970: 142~143,1970
4) CORONELLI, C.; A. VIGEVANI, B. CAVALLERI & G. G. GALLO: Structure determination of pyracrimycin A, a new antibiotic substance. J. Antibiotics 24: 497~502, 1971