Tetrahedron Letters No. 43, pp 3849 - 3852, 1976. Pergamon Press. Printed in Great Britain.

STRUCTURES OF RUGULOVASINE-A AND -B AND 8-CHLORORUGULOVASINE-A AND -B

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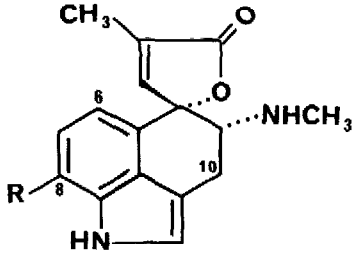
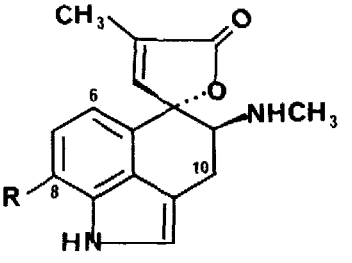
(Received in USA 19 August 1976; received in UK for publication 7 September 1976)

A recent report by Cole, et al.[3] described the isolation of two noncrystalline, chlorinated, toxic metabolites produced by a strain of Penicillium islandicum which was found growing on freshly dug green peanuts. Preliminary physical and chemical analysis indicated that these two new metabolites were indole alkaloids, and were isomeric, but no structures were assigned. We have now investigated the metabolites produced by this fungus in detail, and have found that the crude extract contains two non-chlorinated alkaloids in addition to the two chlorinated compounds previously discovered. We now report the complete structures of these four compounds.

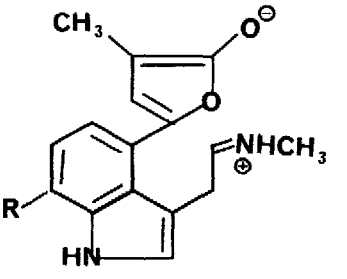
Careful chromatography of the fungal extract on silica gel as described[3] afforded the four compounds in pure form. Spectral data obtained for the two non-chlorinated products, having R_f 0.22 and 0.34 [silica gel PF_{254} plates in chloroform/acetone/methanol (93/7/5)], was identical to that reported for the Ergot alkaloids rugulovasine-A and -B, respectively, isolated from extracts of Penicillium concavo-rugulosum[4]. Direct TLC comparison with authentic material firmly established this



1 R = H
2 R = Cl



3 R = H
4 R = Cl



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identity[5]. Yamatodani, et al. proposed a planar structure for the rugulovasines,[4] and recognized that these compounds are, in fact, diastereomers (cf 1 and 3), but

did not assign stereostructures to the individual compounds. We have settled this point by performing a single crystal X-ray analysis on rugulovasine-A (R_f 0.22), and have found it to have structure 1.

Rugulovasine-A crystallizes as large, clear cubes from chloroform. Preliminary photographs indicated only triclinic symmetry. Precision lattice constants were a = 8.526(2), b = 11.213(3), c = 16.296(3) Å, α = 98.94(5), β = 93.84(5) and γ = 108.96(5)°. Density measurement indicated 4 molecules per unit cell. All unique diffraction maxima with $2\theta \leq 114^{\circ}$ were collected on a Syntex P_{21} diffractometer using graphite monochromated CuK_{α} radiation (1.5418Å). A total of 3283 reflections were measured and after correction for Lorentz, polarization and background effects 2248 (68%) were judged observed ($F_o^2 \geq 3\sigma(F_o^2)$). Intensity statistics indicated a centrosymmetric space group ($P\bar{1}$). Thus, rugulovasine-A is racemic.

The structure was solved by a multisolution, weighted sign determining procedure[6] and refined using full-matrix least-squares techniques[7] to a conventional crystallographic residual of 0.038. Nonhydrogen atoms were corrected for anisotropical thermal motion and hydrogens, for isotropic in the space group $P\bar{1}$.[8]

The asymmetric unit is composed of two molecules of rugulovasine-A and two waters of crystallization. Both molecules in the asymmetric unit represent the same stereostructure. Figure 1 displays a computer generated drawing of one of the molecules of rugulovasine-A in the asymmetric unit. As can be seen the amine N(12) and the lactone O(18) have a cis relationship. All bond distances agree well with generally accepted values. Rugulovasine-B must therefore have structure 3.

We have confirmed the report[4] that the diastereomeric rugulovasines can be interconverted upon warming in polar solvents. This interconversion might be explained by a reverse Mannich reaction of 1 or 3 to the common intermediate 5, which could reclose to either rugulovasine-A or -B. Intermediate 5 is archiral, and this mechanism may also explain why both 1 and 3 are racemic. Since this interconversion is so facile, it is quite possible that either rugulovasine-A or -B, or the lack of optical activity, is an artifact of the isolation procedure.

On the basis of NMR spectral data it is possible to assign the 8-chlororugulovasine-A structure 2 to one of the chlorinated metabolites (R_f 0.27, "Toxin B" in reference 3), and the 8-chlororugulovasine-B structure 4 to the other chlorinated metabolite (R_f 0.44, "Toxin A" in reference 3).[9] Comparison of the characteristically different envelopes of the C-10 and C-11 hydrogens at 3.1δ leads to the assignment of the more polar chlorinated compound to the rugulovasine-A series, and the less polar compound to the rugulovasine-B series. A LIS experiment with $Eu(fod)_3$ reinforced this assignment. In rugulovasine-B and chlororugulovasine-B both the α and β hydrogens at C-10 have identical induced shifts. In rugulovasine-A and chlororugulovasine-A the α and β hydrogens at C-10 have very different induced shifts.

NMR experiments also firmly established the position of chlorine at C-8 in both of the compounds. It is apparent that one of the hydrogens in the benzene ring of rugulovasine-B (3) has been replaced by chlorine, and the remaining two aromatic

hydrogens show ortho coupling.[3] Thus, chlorine must be located at either C-6 or C-8. The LIS experiment with $Eu(fod)_3$ clarified this point. Complexation of the metal was found to occur primarily in the area between the basic nitrogen and the lactone oxygen in 3, and therefore the observed shifts of the benzenoid hydrogens are in the order C-8 < C-7 < C-6. In the chlorinated rugulovasine-B 4, the proton with the largest slope (4.3 ppm/mol), assigned to the one at C-6, is still present. The proton with the smallest slope (1.6 ppm/mol), assigned to the C-8 hydrogen, has disappeared. A similar result was obtained with the rugulovasine-A series, although the aromatic region could not be as cleanly resolved due to lack of material.

Confirming that the halogen has the same position in the two chlorinated metabolites is the observation that these two compounds, like the non-chlorinated products, are interconverted readily in refluxing methanol.[10] To our knowledge, 2 and 4 are the first naturally occurring halogenated Ergot alkaloids to be reported.

Acknowledgement. The work at Fordham University was supported by grants from the National Cancer Institute (CA-12568) and from Eli Lilly and Company. We thank Professor R.W. Franck for helpful discussions.

References

(1) Alfred P. Sloan Foundation Fellow, 1973-75; Camille and Henry Dreyfus Foundation Teacher-Scholar, 1972-77.

(2) Alfred P. Sloan Foundation Fellow, 1975-77; NIH Career Development Awardee, 1975-80.

(3) R.J. Cole, J.W. Kirksey, H.G. Cutler, D.M. Wilson and G. Morgan-Jones, Can. J. Microbiol., 22, 741 (1976).

(4) (a) M. Abe, S. Ohmomo, T. Ohashi, and T. Tabuchi, Agr. Biol. Chem. (Tokyo), 33, 469 (1969); (b) S. Yamatodani, Y. Asahi, A. Matsukura, S. Ohmomo and M. Abe, ibid., 34, 485 (1970).

(5) We are grateful to Dr. S. Yamatodani, Takeda Chemical Industries, for samples of rugulovasine-A and -B hydrochloride.

(6) G. Germain, P. Main, and M.M. Woolfson, Acta Cryst., B24, 274 (1970).

(7) The following crystallographic programs were used: W.R. Busing, K.O. Martin, and H.A. Levy, "ORFLS, A Fortran Crystallographic Least Squares Program", USAEC Report ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965. C. Johnson "ORTEP, A Fortran Thermal-Ellipsoid Plot Program", U.S. Atomic Energy Commission Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.

(8) Fractional coordinates, bond distances, bond angles and observed and calculated structure factors is available as a Supplement to Publication. To obtain a microfiche copy of the Supplement to Publication, contact the Photo Service, Iowa State University, Ames, Iowa 50011; requesting Supplement to Publication for this article and submitting $0.50 in the form of check, cash, or money order Give your name and complete address (including zip code) for mailing.

(9) We wish to correct an error in reference 3. The biological testing data reported for "Toxin B" is actually that for rugulovasine-B (3), and not for a chlorinated metabolite.

(10) The chlorinated compounds also appear to be racemic, and the racemization, like the interconversion may occur via an intermediate such as 5.

Figure 1. A computer generated perspective drawing of rugulovasine-A (1). Hydrogens are not shown for clarity, and only one enantiomer is drawn.