Tetrahedron Letters No. 13, pp 1013 - 1014, 1976. Pergamon Press. Printed in Great Britain.

NEW METABOLITES FROM *ASPERGILLUS TERREUS* RELATED TO THE BIOSYNTHESIS OF ASPULVINONES

Nobutoshi Ojima, Ikuko Takahashi, Kyozo Ogura, and Shuichi Seto*

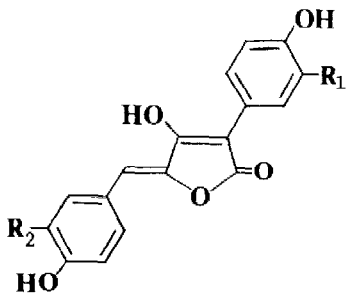
Chemical Research Institute of Non-Aqueous Solutions,
Tohoku University, Katahira 2-1-1, Sendai, Japan

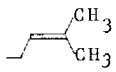
(Received in Japan 19 January 1976; received in UK for publication 17 February 1976)

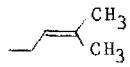
We reported the isolation from *Aspergillus terreus* of seven metabolites including dihydroxypulvinone (1) and its derivatives with an extra hydroxyl group and/or C_5 units.[1,2] Since a report appeared recently describing the isolation of 1 from the same fungus,[3] we wish to propose for avoiding confusion that the name "aspulvinone" be used as the generic name for a series of these metabolites and that aspulvinone A ∿ G be applied to the seven compounds which were designated compound A ∿ G in our previous paper. [1]

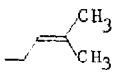
In a subsequent study on the biosynthesis of these compounds, we demonstrated that a cell-free enzyme system from the fungus catalyzed the transfer of the C_5 units from 3,3-dimethylallyl pyrophosphate (DMAPP) to the aryl rings of 1 to give the prenylated derivative (2). However, the occurrence of 2 as a normal metabolite was unknown. This paper describes the isolation of this compound and its biogenetically related compound.

Silica gel chromatography of the ether extracts of the 10 day-old culture filtrate of *Aspergillus terreus* gave two new metabolits, aspulvinone H and aspulvinone I, the former being the major component. Aspulvinone H showed nmr signals at 1.80 (s. 12H), 3.38 (d. 4H, J = 8.0 Hz), 5.40 (t. 2H, J = 8.0 Hz), and 6.48-7.70 ppm (m. 7H), and was identified by the chromatographic and mass spectral comparison with 2 obtained by the enzymatic reaction of 1 with DMAPP. Aspulvinone I, mp 183-185°, showed uv and ir spectra of the aspulvinone type [uv, λ_{max}^{MeOH} nm(log ε): 240(4.07), 326(4.24), 370(4.05); ir, λ_{max}^{KBr} cm^{-1} : 3300, 1720, 1600]. The nmr spectrum showed signals at 1.78 (s. 6H), 3.38 (d. 2H, J = 8.0 Hz), 5.38 (t. 1H, J = 8.0 Hz), and 6.45-7.89 ppm (m. 8H). These data and the mass spectrum (M^+, 364) support that aspulvinone I has the structure 3 or 4.



1 : $R_1 = R_2 = H$; 2 : $R_1 = R_2 =$ 

3 : $R_1 =$  ; $R_2 = H$

4 : $R_1 = H$; $R_2 =$ 

For a further confirmation of the relation between aspulvinone H and aspulvinone I, the enzymatic conversion of the latter to the former was examined. The incubation mixture contained, in a final volume of 1.0 ml, 50 μmol of Tris-HCl buffer, pH 7.0, 10 μmol of $MgCl_2$, 10 μmol of KF, 30 nmol of ^{3}H-labelled DMAPP, 50 nmol of aspulvinone I, and 0.2 mg of enzyme protein prepared as reported.[4] The incubation was carried out at 37° for 1 hr, and the radioactive product obtained from the reaction mixture was identified with aspulvinone H. The yield of the conversion was 68% based on DMAPP.

The enzymatic formation of aspulvinone I was also observed in an early stage of the enzymatic reaction of 1 with ^{3}H-labelled DMAPP. The incubation mixture contained the same as described above except that 1 μmol of 1 and 50 nmol of ^{3}H-DMAPP were employed in place of aspulvinone I and ^{3}H-DMAPP (30 nmol). The radiochromatographic analysis of the products revealed that both aspulvinone H and aspulvinone I were formed in a ratio of *ca.* 3 : 2 after 5 min, and that the ratio increased with the incubation time, the formation of the former being almost exclusive after 25 min.

These results support the biogenetic sequence that aspulvinone H (2) is formed from aspulvinone E (1) *via* aspulvinone I and then cyclized to aspulvinone B and aspulvinone A having the chromane rings. It remains to be defined whether aspulvinone I is 3 or 4.

The amount of aspulvinone H and I produced by the fungus decreases markedly with the age of the culture, and they disappear after 3 weeks when other aspulvinones become dominant.

References

1. N. Ojima, S. Takenaka, and S. Seto, *Phytochemistry*, 12, 2527 (1973).
2. N. Ojima, S. Takenaka, and S. Seto, *Phytochemistry*, 14, 573 (1975).
3. B. T. Golding, R. W. Rickard, and Z. Vanek, *J.C.S. Perkin I*, 1961 (1975).
4. N. Ojima, K. Ogura, and S. Seto, *J.C.S. Chem. Commun.*, 717 (1975).