ALLIXIN, A STRESS COMPOUND FROM GARLIC

Yukihiro KODERA,^{*,a} Hiromichi MATSUURA,^a Susumu YOSHIDA,^a Toshihiko SUMIDA,^a Yoichi ITAKURA,^a Toru FUWA,^a and Hoyoku NISHINO^b

Central Research Laboratories, Wakunaga Pharmaceutical Co., Ltd.,^a Shimokotachi 1624, Koda-cho, Takatagun, Hiroshima 729-64, Japan, and Kyoto Prefectural University of Medicine,^b Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, Japan

A new stress compound, named allixin, was isolated from garlic, bulbs of Allium sativum L. and the structure was identified by NMR and MS spectrometry as 3-hydroxy-5-methoxy-6-methyl-2-pentyl-4H-pyran-4-one.

KEYWORDS Allium sativum; garlic; Liliaceae; stress compound; allixin; 4-pyrone derivative

Garlic, bulbs of Allium sativum L., has been used world-wide as food and medicine. There are many reports on characteristic sulfur components of garlic as alliin, allicin, and volatile oils.¹⁾ Recently, we reported the isolation, structure determination, and antifungal activities of steroidal glycosides from garlic and related plants.²⁾ The present paper deals with the isolation and structure elucidation of a new stress compound.

A methanolic extract of garlic cloves was extracted with CHCl_3 and the resulting extract was chromatographed on silica gel and reversed-phase highly porous polymer to give a new phenolic compound (1), named allixin, $\text{C}_{12}\text{H}_{18}\text{O}_4$, colorless needles (from aqueous MeOH), mp 80–81°C. The EI-MS spectrum of 1 showed a molecular ion at m/z 226 and the ^1H - and ^{13}C -NMR spectra of 1³⁾ indicated the presence of methyl, n-pentyl, methoxyl, and hydroxyl groups. These assignments were confirmed by the analysis of the ^1H - ^1H and ^1H - ^{13}C 2D-COSY spectra. In addition, the signals due to one ketone and four quarternary carbons appeared in the ^{13}C -NMR spectrum of 1. The IR spectrum of 1 exhibited a band attributable to α , β -unsaturated ketone at 1660 cm^{-1} (KBr) and the UV spectrum of 1 showed a UV_{max} at 279 nm (ϵ 10500, in MeOH). These results led to the formulation of 1 as tetrasubstituted 4-pyrone having methyl, n-pentyl, methoxyl, and hydroxyl groups.

The locations of these groups in 1 were as follows. In the 2D-NOESY experiment of 1, NOE was observed between methyl and methoxyl groups, indicating that these groups may be located at neighboring positions. The ^{13}C -NMR spectra demonstrated that on going from maltol (2),⁴⁾ 3-hydroxy-2-methyl-4H-pyran-4-one, to its acetate (3), C-2 and C-5 were evidently deshielded, but C-3 and C-4 were shielded, while C-6 remained almost unshifted. The similar acetylation shifts were also observed for 1 and its acetate (4), suggesting that the hydroxyl group was bonded to C-3 of 1. On inspection of these acetylation shifts, the carbon signals of 1 and 4 were assigned as shown in Table I. The location of methoxyl group was determined to be C-5 by comparison of the ^{13}C -NMR signals of 1 with those of 3-methoxy-2-methyl-4H-pyran-4-one (5).

Accordingly, the structure of 1 was established to be 3-hydroxy-5-methoxy-6-methyl-2-pentyl-4H-pyran-4-one (Fig. 1).

Compound 1 was produced in garlic clove damaged by burning or treatment with HgCl_2 ,⁵⁾ H_2O_2 ,⁶⁾ cellulase, or pectinase⁷⁾ (Table II). However, compound 1 was rarely detected in the extract of control garlic clove. Figure 2 shows the time course of production of 1 by the treatment with HgCl_2 . It is noteworthy that 1 is a new stress compound from garlic.

The antimicrobial activity of 1 was very weak against Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger, and Escherichia coli.

Other biological activities of 1 will be reported elsewhere.

Table I. ^{13}C -NMR Chemical Shifts of 4-Pyrones(1 - 5) in CDCl_3

	1	4	2	3	5
C-2	150.3	160.3	149.4	158.9	159.2
C-3	141.9	137.5	143.2	138.2	145.5
C-4	169.5	167.9	173.1	167.2	174.1
C-5	141.9	144.3	113.2	116.3	117.0
C-6	158.0	157.8	154.0	154.2	153.5
C-7	15.0	14.8	14.2	14.6	14.4
C-1'	26.3	26.2			
C-2'	28.3	28.7			
C-3'	31.2	31.1			
C-4'	22.3	22.2			
C-5'	13.9	13.8			
OCH_3	60.1	60.1			59.8
COCH_3		20.3		19.9	
COCH_3		169.2		171.7

Fig. 1

Table II. Production of 1 by Stresses

	Content($\mu\text{g/g}$ (wet weight))
Control	Trace
1% HgCl_2	19
30% H_2O_2	79
Cellulase	14
Pectinase	130
Burn	55

Garlic cloves were washed successively with H_2O and sterilized with 0.1% benzalkonium chloride, 70% EtOH and 10% formaldehyde. Then the surface of the cloves were treated with reagents (20 μl /clove in the case of 1% HgCl_2) or damage shown in the Table II, and the cloves were incubated at room temperature. After 15 days, the samples (5-15 g) were extracted with MeOH (50 ml), the supernatant (10-20 μl) was applied to HPLC analysis (see Fig. 3).

Fig. 2. Time Course Experiment of 1 Accumulation
o: Control ●: 1% HgCl_2

Fig. 3. HPLC Pattern of MeOH-Extract of Garlic by Treatment with HgCl_2
HPLC Condition: Column, TSK GEL ODS80TM; Mobile Phase, 0.05 M phosphate buffer (pH 3.0)-MeOH (28:72); Flow Rate, 1.0 ml/min; Detection, 280 nm, 0.005 Afs

ACKNOWLEDGEMENT We are grateful to Prof. O. Tanaka and Dr. M. Miyakoshi, Hiroshima University, for elemental analysis.

REFERENCES AND NOTES

- 1) G. Vernin, J. Metzger, D. Fraisse, and C. Scharff, *Planta Medica*, 52, 96 (1986) and references cited therein.
- 2) H. Matsuura, T. Ushiroguchi, Y. Itakura, N. Hayashi, and T. Fuwa, *Chem. Pharm. Bull.*, 36, 3659 (1988); T. Morita, T. Ushiroguchi, N. Hayashi, H. Matsuura, Y. Itakura, and T. Fuwa, *ibid.*, 36, 3480 (1988); H. Matsuura, T. Ushiroguchi, Y. Itakura, and T. Fuwa, *ibid.*, accepted.
- 3) $^1\text{H-NMR}$ (270 MHz, CDCl_3): δ 0.96 (3H, t, $J = 6.9$ Hz), 1.33 (2H x 2, m), 1.66 (2H, tt, $J = 7.3$ Hz), 2.33 (3H, s), 2.67 (2H, t, $J = 7.3$ Hz), 3.88 (3H, s), 6.50 (1H, br s, OH).
- 4) C. A. Kingsbury, M. Clifton, and J. H. Looker, *J. Org. Chem.*, 41, 2777 (1976).
- 5) I. Uritani, *Kagaku to Seibutsu*, 12, 546 (1974).
- 6) A. Murai, Y. Yoshizawa, K. Sato, T. Hasegawa, and T. Masamune, The 16th International Symposium on the chemistry of Natural Products, Kyoto, May, 1988.
- 7) P. Albersheim and A. G. Darvill, *Scientific American*, 1985, 253.

(Received April 12, 1989)