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, Takata-gun, 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, $C_{12}H_{18}O_4$, 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 1[3] 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).

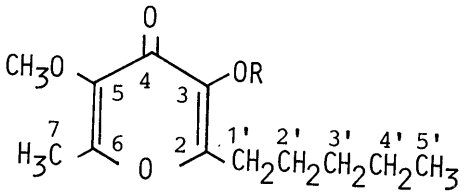
Compound 1 was produced in garlic clove damaged by burning or treatment with $HgCl_2$,[5] H_2O_2,[6] cellulase, or pectinase[7] (Table II). However, compound 1 was rarely detected in the extract of control garlic clove. Figure 2 shows the time course of production of 1 by the treatment with $HgCl_2$. 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 nigar, and Escherichia coli.

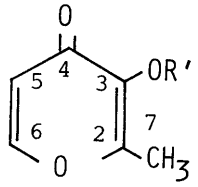
Other biological activities of 1 will be reported elsewhere.

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
$CO\underline{C}H_3$		20.3		19.9	
$\underline{C}OCH_3$		169.2		171.7	



1: R= H
4: R= Ac



2: R'= H
3: R'= Ac
5: R'= Me

Fig. 1

Table II. Production of 1 by Stresses

	Content(μg/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).

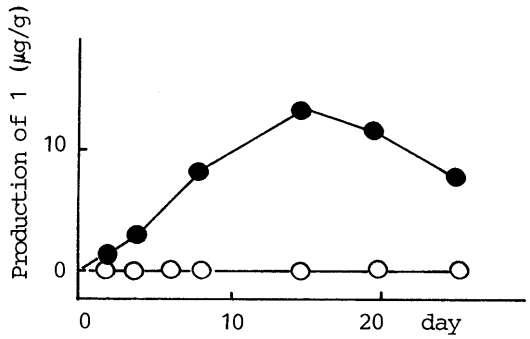


Fig. 2. Time Course Experiment of 1 Accumulation
o: Control ●: 1% $HgCl_2$

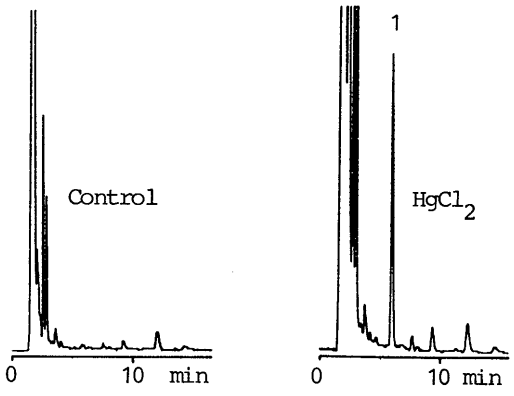


Fig. 3. HPLC Pattern of MeOH-Extract of Garlic by Treatment with $HgCl_2$
HPLC Condition: Column, TSK GEL ODS80TM; Mobile Phase, 0.05 M phosphate buffer (pH 3.0)-MeOH (28:72); Flow Rate, 1.0 ml/min; Detection, 280 nm, 0.005 Aufs

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: ^{1}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= 7.3Hz), 3.88(3H, s), 6.50(1H, br s, OH).
4) C. A. Kingsbury, M. Cliffton, 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)