Communications to the Editor

[Chem. Pharm. Bull. 33(11)5141—5143(1985)]

NOVEL BIOTRANSFORMATION OF A 2-PYRONE TO A SUBSTITUTED BENZOIC ACID

Ikuo Sakurai, Harumi Suzuki, Sakae Shimizu and Yuzuru Yamamoto*
Fuculty of Pharmaceutical Sciences, Kanazawa University,
Takaramachi 13-1, Kanazawa 920, Japan

It was found that Macrophoma commelinae(IFO 9570) had an ability to transform 5-acetyl-4-methoxy-6-methyl-2-pyrone(1) to 4-acetyl-3-methoxy-5-methylbenzoic acid(2). This biotransformation was investigated using ^{13}C- and ^{14}C-labeled compounds. It is likely that 2 is formed by condensation of the added 2-pyrone and a catabolic pyruvate.

KEYWORDS——Macrophoma commelinae; fungus; 2-pyrone; substituted benzoic acid; pyruvate; aromatic ring formation

In a previous paper,[1] we reported that four new metabolites, named macommelin and its congeners, were isolated from Macrophoma commelinae(IFO 9570). The chemical structures elucidated were unique in having an alkyl group at the C-5 position of the 2-pyrone ring. The biogenetic investigation is now proceeding at the whole cell and cell free levels. Meanwhile, in experiments on feeding the metabolites and several potential intermediates, it was unexpectedly observed that 5-acetyl-4-methoxy-6-methyl-2-pyrone(1) was trahsformed to a substituted benzoic acid(2) in the resting cell. These two compounds are not metabolites in normal culture. A similar relationship seems to exist between pyrenocins[2] and pyrenochaetic acids[3] from Pyrenochaeta terrestris, whose biosynthesis has not been investigated to date. Here we describe this interesting biotransformation.

Compound 1[4] (220 mg) was administrated to the washed M. commelinae cells in 1 liter of 0.01 M phosphate buffer (pH 6.0) after incubation for 10 days. After further standing for 10 days, an acidic metabolite[5] was formed in a fairly good yield (73%). It was identified as 4-acetyl-3-methoxy-5-methylbenzoic acid(2) from spectral data and by comparison with an authentic sample.[6]

When [4-methoxy-^{14}C] 1 (50.1 μCi/mmol)[7] was administered, 68% of the radioactivity was incorporated into 2 and its specific activity did not show a decline (51.3 μCi/mmol). [3-^{13}C] and [5,7,9-^{13}C] 1 were synthesized from [2-^{13}C]malonic acid and [1,3-^{13}C]acetone, respectively.[8] These compounds were individually administered and the ^{13}C-NMR spectra of the derived 2 were measured. As shown in Table I, the C-2 of 2 was derived from C-3 of 1, and C-4, C-7 and C-9 of 2 were derived from the corresponding C-5, C-7 and C-9 of the added 1 without the bond cleavage. This was indicated by the similar long-range ^{13}C-^{13}C couplings observed in isolated 2 and added 1 ($^2J_{C-5,C-9}$=13.7 Hz, $^2J_{C-5,C-7}$=3.9 Hz).

In a feeding experiment with a mixture of non-labeled 1 and [1,2-^{13}C]acetate, C-1, C-6 and C-11 of 2 were enriched and a ^{13}C-^{13}C coupling (^1J=72.1 Hz) was observed

Table I. ^{13}C-NMR Spectra of 2 Derived from ^{13}C-Labeled Precursors

Carbon No. of 2	δ_C [a] (ppm)	Enhancement [b] [3-^{13}C] 1	Enhancement [b] [5,7,9-^{13}C] 1
7	18.9	1.0	4.4(2.0 Hz) [c]
9	31.8	1.2	4.4(12.7 Hz)
10	56.2	1.0	1.0
2	110.3	5.7	1.0
6	125.1	0.7	0.7
1	132.8	0.5	0.5
5	136.0	1.4	0.7
4	136.2	1.2	8.8(12.7, 2.0 Hz)
3	157.0	0.8	1.0
11	167.4	___ [d]	___
8	204.1	1.7	0.5

a) Relative to internal Me_4Si in $(CD_3)_2CO$. b) Ratio of the signal intensity for enriched and naturally abundant 2 that is normalized for the C-10 signal. c) Long-range ^{13}C-^{13}C coupling observed. d) Signal for C-11 of unlabeled 2 is so weak that the enhancement is not calculated.

between C-1 and C-11. The radioactive 2 obtained by the experiment using [1-^{14}C]-acetate and non-labeled 1, was degradated to cold 2-methoxy-6-methylacetophenone and radioactive CO_2 by treatment with Cu powder in boiling quinoline. Similarly, the radioactivity of added [1-^{14}C]pyruvate was found to be incorporated only into C-11 of 2. Malic acid was also incorporated into 2 with the loss of one carboxyl moiety. The results of incorporation are summarized in Table II. [3-^{14}C]Pyruvate exhibited the highest incorporation ratio (8.0%) of all.

Table II. Incorporation of ^{14}C-Labeled Precursors into 2

Precursor	Incorporation (%)
Sodium [1-^{14}C]acetate	1.2*
Sodium [1-^{14}C]pyruvate	1.5*
Sodium [3-^{14}C]pyruvate	8.0
L-[U-^{14}C]Malic acid	5.2

* The radioactivity is specifically distributed in the carboxyl carbon of 2 (see text).

It is likely that the acetate and malate are incorporated into 2 via pyruvate.[9] As shown in Chart 1, this may be followed by ring formation, decarboxylation in the C-2 of 1 and dehydration (aromatization). Although many processes of fungal aromatic metabolites being degraded to compounds containing an oxygen atom in the ring are known, this reversed aromatic ring formation from 2-pyrone is the first known example of such a phenomenon, to our knowledge.

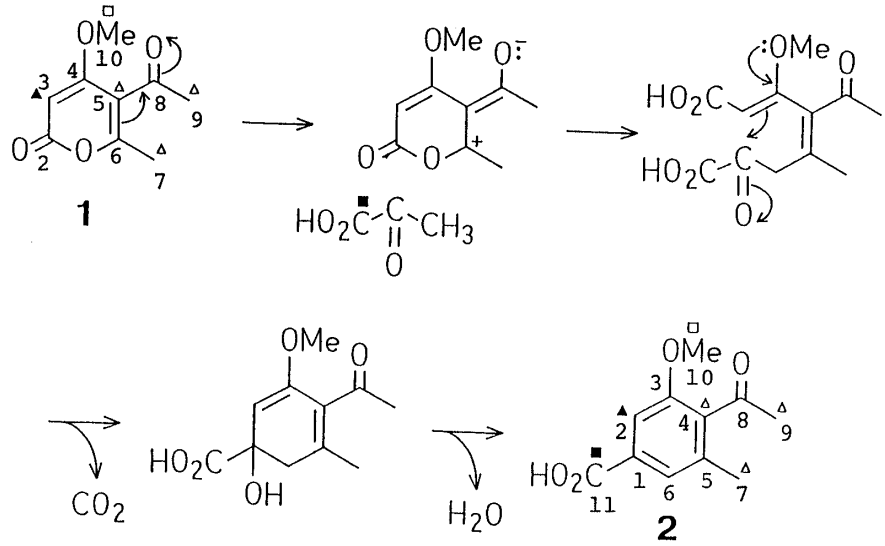

Chart 1

ACKNOWLEDGEMENT We are grateful to the Institute for Fermentation, Osaka, for supplying the IFO strain, and to Dr. S. Sakamura for supplying a synthesized authentic sample. We also thank Mr. Y. Itatani for the elemental analyses and spectral measurements. This work was partly supported by a Grant-in-Aid (No. 58370041) from the Ministry of Education, Science and Culture of Japan.

REFERENCES AND NOTES

1) S. Shimizu, I. Sakurai and Y. Yamamoto, Chem. Pharm. Bull., 31, 3781 (1983).
2) a) H. Sato, K. Konoma, S. Sakamura, A. Furusaki, T. Matsumoto and T. Matsuzaki, Agric. Biol. Chem., 45, 795 (1981); b) S. A. Sparace, J. B. Mudd, B. A. Burke and A. J. Aasen, Phytochemistry, 23, 2693 (1984).
3) H. Sato, K. Konoma and S. Sakamura, Agric. Biol. Chem., 45, 1675 (1981).
4) 1 was prepared by the condensation[a] of acetylacetone with malonyl chloride and subsequent methylation[b] with NaH/Me_2SO_4. a) M. A. Butt and J. A. Elvidge, J. Chem. Soc., 1963, 4483; b) E. Suzuki, B. Katsuragawa and S. Inoue, Synthesis, 1978, 144.
5) 2: colorless plates, mp 179-182°C (from C_6H_6). Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.58; H, 5.71. MS m/z: 208(M^+), 193, 150, 43. UV λ_{max}^{EtOH}nm: 250, 304. IR $\nu_{max}^{KBr}cm^{-1}$: 3005-2400, 1690, 1573, 1461. ^{1}H-NMR (in $(CD_3)_2CO$) δ: 2.25(s, CH_3), 2.45(s, $COCH_3$), 3.93(s, OCH_3), 7.49(bs, Ar-H), 7.52 (bs, Ar-H), 8.5(bs, COOH).
6) The acid was obtained by alkaline hydrolysis of the ethyl ester supplied by S. Sakamura; A. Ichihara, K. Murakami and S. Sakamura, Agric. Biol. Chem., 48, 833 (1984).
7) [4-Methoxy-^{14}C] 1 was prepared by methylation of 5-acetyl-4-hydroxy-6-methyl-2-pyrone with $^{14}CH_3I/Ag_2O$.
8) [3-^{13}C] 1 was prepared from [2-^{13}C]malonate as previously described.[4] [5,7,9-^{13}C] 1 was prepared from [1,3,5-^{13}C]acetylacetone which was synthesized from [1,3-^{13}C]acetone and acetic anhydride; C. E. Denoon, "Organic Syntheses," Col. Vol. II, John Wiley and Sons, Inc., New York, 1943, pp. 907.
The purchased ^{13}C-acetone and ^{13}C-malonate (both 90 atom %) were used to synthesize after 10-fold dilution.
9) J. E. Holenstein, A. Stoessl, H. Kern and J. B. Stothers, Can. J. Chem., 62, 1971 (1984).

(Received September 9, 1985)