Notes

TWO THIOLACTONES FROM *STREPTOMYCES* TÜ 2476

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In the course of our screening of new secondary metabolites, two thiolactones have been isolated from the culture of the *Streptomyces* strain Tü 2476. In this report, the isolation, characterization, and structure of the two thiolactones are reported.

The *Streptomyces* strain Tü 2476, isolated from a soil sample which has been collected near Kanchanabari (West Thailand), was cultured at 27°C for 4 days in 500-ml Erlenmeyer flasks containing 100 ml of medium composed of 2% mannitol and 2% soybean meal (initial pH 7.5). The final pH 7.7 of the culture broth (2.5 liters) was adjusted to pH 7.0. The mycelium was filtered off and extracted with MeOH (3×2 liters) and the culture filtrate was extracted with EtOAc (5×1.5 liters). The extracts were evaporated and the residues combined. They were suspended in water and extracted with ether (3× 100 ml). The EtOAc phases were combined and precipitated by hexane.

The substances obtained from the ethereal extract were dissolved in MeOH - Me_2CO (1: 1) and treated with activated charcoal. The solvent was evaporated and the residue chromatographed on silica gel 60 F_{254} plates (2 mm) in $CHCl_3$ - Me_2CO - MeOH, 90: 9: 1. Two isolated fractions were then purified by column chromatography on Sephadex LH-20 in MeOH. Total amounts obtained were 27 mg of compound B_4 (faster moving) and 43 mg of compound B_3, which was identified as OAc-*o*-aminophenol (EI-MS, ^1H and ^{13}C NMR).

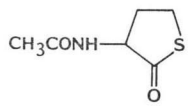
The precipitate from EtOAc extract was subjected to TLC on silica gel 60 F_{254} (2 mm) in benzene - $CHCl_3$ - EtOAc - MeOH, 7: 7: 1: 0.7. The main fraction after crystallization from benzene yielded 24 mg of compound B_2.

Compound B_2: White needles, mp 142°C (benzene). *Anal* found: C 45.42, H 5.59, N 8.79; calcd for $C_6H_9NO_2S$ (159.21): C 45.26, H 5.69, N 8.79. EI-MS (35°C) *m/z* (% of relative intensity, elemental composition, *m/z* of daughter ions): 159 (1, $C_6H_9NO_2S$, 131), 131 (53, C_5H_9NOS, 103, 98, 88), 103 (13, C_3H_5NOS), 98 (6, C_5H_8NO, 56), 88 (22, C_3H_6NS, 61, 56, 43), 61 (27, C_2H_5S), 56 (56, C_3H_6N), 43 (100, C_2H_3O+ C_2H_5N, 1: 1). ^1H NMR ($CDCl_3$, 59.797 MHz, 25°C): 2.05 (s, 3H), 2.06 (m, 1H), 2.93 (m, 1H), 3.02~3.67 (m, 2H), 4.59 (ddd, J=6.4, 6.4, and 12.7 Hz, 1H), 6.45 (br s, 1H). ^{13}C NMR ($CDCl_3$, 15.036 MHz, 25°C): 23.0 (q), 27.5 (t), 31.7 (t), 59.4 (d),170.8 (s), 205.8 (s).

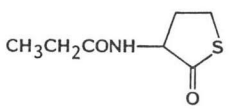
Compound B_4: White needles, mp 124°C (EtOAc). *Anal* found: C 48.09, H 5.99, N 7.92; calcd for $C_7H_{11}NO_2S$ (173.24): C 48.53, H 6.40, N 8.08. EI-MS (35°C) *m/z* (% of relative intensity, elemental composition, *m/z* of daughter ions): 173 (3, $C_7H_{11}NO_2S$, 145, 126), 145 (68, $C_6H_{11}NOS$, 117, 112, 88), 126 (2, $C_6H_8NO_2$), 117 (11, C_4H_7NOS, 61), 112 (4, $C_6H_{10}NO$, 57), 89 (26, C_3H_7NS), 88 (52, C_3H_6NS, 61), 61 (39, CH_3NS), 57 (100, C_3H_5O+C_3H_7N, 10: 1), 56 (68, C_3H_6N), 43 (37, C_2H_5N), 29 (87, C_2H_5). ^1H NMR ($CDCl_3$, 59.797 MHz, 25°C): 1.16 (t, J=7.3 Hz, 3H), 1.88 (m, 1H), 2.29 (q, J=7.3 Hz, 2H), 2.97 (m, 1H), 3.11 (d, J=11.6 Hz, 1H), 3.52 (dd, J=4.3 and 11.6 Hz, 1H), 4.52 (ddd, J=6.1, 6.7, and 12.8 Hz, 1H), 5.90 (br s, 1H). ^{13}C NMR ($CDCl_3$, 15.036 MHz, 25°C): 9.5 (q), 27.6 (t), 29.4 (t), 32.1 (t), 59.8 (d), 174.3 (s), 205.7 (s).

The two compounds B_2 and B_4 are evidently homologues, according to their molecular formulas. They share several common features: ions *m/z* 88, 61, 56 and 43 in their mass spectra, an ABCDXY spin system corresponding to an arrangement $-NHCHCH_2CH_2-$ in ^1H NMR, five signals with close chemical shifts and the same multiplicity in ^{13}C NMR. The fragmentation pattern upon electron impact is also similar; the expulsion of carbon monoxide from the mo-

lecular ion is followed by three different pathways — losses of C_2H_4, SH or RCO (R=CH_3 or C_2H_5, respectively). ^1H NMR spectra show that there is an acetyl group in B_2 but an ethyl one in B_4. It can be concluded that both compounds have the same C_5H_6NOS nucleus and differ in the side chain only. The two most downfield signals in the ^{13}C NMR spectra might be due either to the C=O or C=S type atoms. Since both oxygen atoms present are lost upon electron impact together with carbons, the latter possibility is excluded. The balance of oxygen atoms also resolves the ambiguity in the assignment of signal around 170 ppm (ester, lactone, or amide) in favor of the amide. Both ^{13}C NMR spectra differ in the chemical shifts of the high field carbonyl and the side chain carbons. Therefore, the signal at 170.8 (or 174.3) ppm is assigned to the amide group carbonyl to which the side chain is attached. The methine carbons of both $-NHCHCH_2CH_2-$ systems resonate at 59.4 and 59.5 ppm in B_2 and B_4, respectively (found by selective hetero-nuclear decoupling). By the same method it was found that terminal methylenes resonate at 27.5 and 27.6 ppm. Therefore, the carbonyl group is attached to the methine and the sulfur atom is bonded to the methylene. The final formulas **I** for B_2 and **II** for B_4 show that both compounds are derived from homocysteine thiolactone. This hypothesis was proved by a synthesis which provided **I** identical by mp, ^1H and ^{13}C NMR, and mass spectra with our isolate B_2.



I



II

N-Acetylhomocysteine thiolactone is a known compound and is commercially available. It is included in some medical preparations[1], and is also used as a reagent for insolubilizing antibodies[2]. Both our substances did not show any antimicrobial activity in the diffusion assay against *Bacillus subtilis, Bacillus brevis, Escherichia coli, Clostridium pastorianum, Streptomyces viridochromogenes, Mucor miehei,* and *Botrytis cinerea.* Very recently two other five-membered thiolactones with antimicrobial activity have been reported[3~5].

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