Abstract

Isoshamixanthone (1), a new stereoisomeric pyrano xanthone together with the previously known fungal metabolites, epiisoshamixanthone (2), sterigmatocystin (3), arugosin C (4), norlichexanthone (5), diorcinol (6), ergosterol, and methyllinoleate were obtained from the endophytic fungal strain Aspergillus sp. ASCLA isolated from leaf tissues of the medicinal plant Callistemon subulatus. The chemical structure of the new xanthone (1) was elucidated by extensive 1D, 2D NMR, and ESI HR mass measurements, and by comparison with literature data. The constitutions and absolute configurations of 1 and epiisoshamixanthone (2) were additionally confirmed by X-ray crystallography. Compounds 1,2 were evaluated for their potential anticancer activity using the human cervix carcinoma cell line (KB-3-1). The antimicrobial activities of the fungal extract and compounds 1,2 were studied using a panel of pathogenic microorganisms as well.
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Table S1. Physico-chemical properties of isoshamixanthone (1) and epiisoshamixanthone (2)

|          | 1                 | 2                 |
|----------|-------------------|-------------------|
| Appearance | yellow solid     | yellow solid     |
| $R_f$    | 0.54$^b$         | 0.56$^b$         |
| UV (254 nm) | Strong UV absorbing | Strong UV absorbing |
| UV (366 nm) | -                 | -                 |
| Staining with anisaldehyde/ sulfuric acid and heating | dark blue | dark blue |
| Molecular formula | C$_{25}$H$_{26}$O$_5$ (406) | C$_{25}$H$_{26}$O$_5$ (406) |
| (+)-ESI-MS: m/z (%) | 429 ([M+Na]$^+$) | 429 ([M+Na]$^+$) |
| (+)-HRESIMS: m/z | -                 | -                 |
| Found     | 429.16738 [M+Na] | 429.16738 [M+Na] |
| Calcd.    | C$_{25}$H$_{26}$O$_5$Na | C$_{25}$H$_{26}$O$_5$Na |
| $[\alpha]_{D}^{20}$ | +109.3° (c = 0.14, CHCl$_3$) | +130.3° (c = 0.11, CHCl$_3$) |

$^a$Silica gel G/UV254, $^b$(cyclohexane/80% DCM)

Table S2: $\textsuperscript{13}$C (125 MHz) and $\textsuperscript{1}$H (500 MHz) NMR data of isoshamixanthone (1) and epiisoshamixanthone (2) in CDCl$_3$

| Position | Position | $\delta_{C}$ [ppm] | $\delta_{H}$ [ppm] (m, J [Hz]) | $\delta_{C}$ [ppm] | $\delta_{H}$ [ppm] (m, J [Hz]) |
|----------|----------|--------------------|---------------------------------|--------------------|---------------------------------|
| 1        | 14       | 158.7              | -                               | 158.7              | -                               |
| 1-OH     | 15       | -                  | 12.92 (s, 1H)                   | 12.90 (s, 1H)      | -                               |
| 2        | 2        | 122.6              | -                               | 122.6              | -                               |
| 3        | 4        | 136.7              | 7.40 (d, 8.5, 1H)               | 136.6              | 7.40 (d, 8.5, 1H)               |
| 4        | 5        | 105.7              | 6.79 (d, 8.5, 1H)               | 105.7              | 6.79 (d, 8.5, 1H)               |
| 5        | 6        | 119.3              | 7.17 (d, 1.0, 1H)               | 119.4              | 7.19 (d, 1.0, 1H)               |
| 6        | 7        | 138.3              | -                               | 138.4              | -                               |
| 7        | 8        | 149.3              | -                               | 149.1              | -                               |
| 8        | 9        | 121.0              | -                               | 121.6              | -                               |
| 9        | 10       | 116.9              | -                               | 116.8              | -                               |
| 10       | 11       | 184.5              | -                               | 184.3              | -                               |
| 11       | 12       | 108.8              | -                               | 108.8              | -                               |
| 12       | 13       | 153.8              | -                               | 153.8              | -                               |
| 13       | 14       | 152.3              | -                               | 152.1              | -                               |
| 15       | 16       | 64.5               | 4.34 (ddd, 10.8, 3.5, 1.3, 1H), 4.27 | 64.0              | 4.39 (ddd, 10.1, 3.4, 1.5, 1H), (dd, 10.9, 3.0, 1H) |
| 16-OH    | 17       | -                  | 5.35 (ddt, 5.0, 3.3, 1.6, 1H)   | 61.3              | 5.43 (dd, 3.8, 1.4, 1H)         |
| 17       | 18       | 27.2               | 3.32 (d, 7.3, 2H)               | 27.1              | 3.32 (d, 7.4, 2H)               |
| 18       | 19       | 121.8              | 5.27 (tdq, 7.3, 3.0, 1.5, 1H)   | 121.8              | 5.27 (ttq, 7.3, 1.4, 1H)        |
| 19       | 20       | 133.3              | -                               | 133.3              | -                               |
| 20       | 21       | 25.8               | 1.69 (d, 1.5, 3H)               | 25.8              | 1.69 (m, 3H)                    |
| 21       | 22       | 17.8               | 1.68 (s, 3H)                    | 17.8              | 1.68 (s, 3H)                    |
| 22       | 23       | 17.4               | 2.27 (d, 0.9, 3H)               | 17.5              | 2.29 (d, 0.9, 3H)               |
| 23       | 24       | 142.6              | -                               | 142.3              | -                               |
| 24       | 25       | 112.3              | 4.73 (t, 1.5, 1H), 4.51 (s, 1H) | 111.5              | 4.99 (d, 1.3, 1H), 4.71 (s, 1H) |
| 25       |          | 22.6               | 1.78 (s, 3H)                    | 22.5              | 1.92 (s, 3H)                    |
Table S3. Antimicrobial activities of ASCLA extract and compound 1-2 using agar diffusion method (mm diameter)

| Ext/Comp         | EC  | BS  | Psa a | ML b | Stw c | Sta d | Psa eat | Ca b | Sac f | Be g | BS k | An l |
|------------------|-----|-----|-------|------|-------|-------|---------|------|-------|------|------|------|
| ASCLA extract    | -   | 8   | 8     | -    | -     | 15    | 20      | 20   | 16    | ND   | ND   | 15   |
| 1                | -   | -   | -     | -    | -     | 13    | 16      | 15   | 10    | 11   | 14   | -    |
| 2                | -   | -   | -     | -    | -     | 12    | 12      | 15   | 15    | -    | 11   | -    |
| Gentamycin       | 18  | 23  | 19    | 21   | 21    | 17    | 22      | 18   | 17    | 23   | 20   | 18   |

a E. coli DSMZ 1058; b Bacillus subtilis DSMZ 704; c Pseudomonas agarici DSMZ 11810; d Micrococcus luteus DSMZ 1605; e Staphylococcus warneri DSMZ 20036; f Staphylococcus aureus ATCC 6538-P; g Pseudomonas aeruginosa ATCC 27853; h Candida albicans ATCC 10231; i Saccharomyces cerevisiae ATCC 9080; j Bacillus cereus ATCC 11778; k Bacillus subtilis ATCC 6633; l Aspergillus niger; (-): not active; (ND): not detected (i.e. not measured)

**Figure S2:** Neighbour-joining constructed tree of the fungal strain ASCLA, based on its aligned 18S rRNA gene sequence using MEGA 7 program.

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Figure S19: HMBC spectrum (CDCl$_3$, 500 MHz) of epiisohamixanthone (2)
Figure S20: NOESY spectrum (CDCl₃, 500 MHz) of epiisoshamixanthone (2)