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The structure of testacein, a new hybrid polyketide-sesquiterpene metabolite from the lichen *Notoparmelia testacea*

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# The structure of testacein, a new hybrid polyketide-sesquiterpene metabolite from the lichen *Notoparmelia testacea*

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**Abstract**
Testacein has been isolated from the lichen *Notoparmelia testacea* and its structure established by mass spectrometry and NMR spectroscopy.

**Introduction**
Testacein was first detected in the lichens *Notoparmelia testacea* (Stirt.) A.Crespo, Ferencova & Divakar and *N. subtestacea* (Hale) A.Crespo, Ferencova & Divakar (Hale 1987, as unknown #27) and subsequently found in other lichen genera including *Pyxine* (Kalb 2004; Elix 2009), *Heterodermia* (Elix 2010) and *Buellia* (Elix & Kantvilas 2013). Although testacein could readily be characterized by thin-layer and high-performance liquid chromatography (Hale 1987; Elix 2014), its structure remained unknown. This paper describes the structural elucidation of testacein. Methods are as described in Elix *et al.* (2019)

**Extraction of *Notoparmelia testacea***
The lichen *Notoparmelia testacea* was collected in the Otepatotu Scenic Reserve, Outer Banks Peninsula, South Island, New Zealand, 43°45'S, 173°10'E, 720 m alt., on dead wood at margin of cool temperate rainforest, *J.A. Elix 7716*, 30.iii.1980 (CANB).

The dried lichen thallus (7.3 g) was extracted in a Soxhlet extractor with anhydrous diethyl ether (300 mL) for 48 h. The extract was filtered and the solid [salazinic acid] discarded. The filtrate was concentrated to 30 mL and filtered again and the solid discarded. The final filtrate was concentrated to dryness to yield 111.6 mg of a pale brown oil. A portion (38.3 mg) of this oil was purified by column chromatography over silica gel using 60% ethyl acetate/light petroleum [bp 60–80 °C] as eluent. The major band was collected from the chromatographic column and concentrated to afford testacein (1) (19.6 mg) as a pale yellow oil, $[\alpha]_D^{25} = -8.6$ (c 0.12, $(CH_3)_2CO$).

**Structural elucidation of testacein**
Testacein (1) exhibited *m/z* $[M+Na]^+$ 497.2157 on high resolution ESIMS with a sodiated adduct ion corresponding to $C_{26}H_{34}O_8Na^+$, thus establishing the molecular formula of testacein as $C_{26}H_{34}O_8$. Testacein was found to be unstable in $CDCl_3$ solution, forming an insoluble precipitate, but was stable in $(CD_3)_2CO$ solution. Assignments in the $^1$H-NMR spectrum of (1) are summarized in Table 1 and assignments in the $^{13}$C-NMR spectrum in Table 2.

Spectroscopic evidence showed that testacein possesses the structure (1), similar to the known fungal metabolites pestalotiopene A (2) (Hemberger *et al.* 2013) and pestalotiopene C (3) (Hammerschmidt *et al.* 2014).

In the HSQC spectrum of (1), four C signals (δ 21.8, 33.8, 17.4, 21.2) were strongly associated with four singlet $CH_3$ proton signals (δ 0.84, 0.91, 1.19, 1.63), indicating the presence of four quaternary methyl groups in this compound. A carbon signal (δ 64.0) was associated with a singlet proton signal (δ 4.17, 3H), as expected for a methoxy group in (1). The presence of two CHO groups in (1) was supported by the association between the C signals (δ 206.2, 194.4) and singlet proton signals (δ 9.93, 10.30), respectively.

A comparison of the $^1$H and $^{13}$C NMR resonances of the aromatic moiety and associated phthalide ring in testacein with those of cyclopaldic acid (4) (Achenbach *et al.*, 1982), pestalotiopene A (2) (Hemberger *et al.* 2013) and pestalotiopene C (3) (Hammerschmidt *et al.* 2014), revealed the close analogy of the respective chemical shifts and indicated the presence of the substructure (5) in (1). Observed correlations in the gHMBC spectrum of testacein (1) (Figure 2) confirmed the presence of this substructure.

The observed NOESY correlations for (1) (Figure 3) indicated that the aliphatic CHO (δ 9.94) and three $CH_3$ groups (δ 0.91, 1.19 and 1.63) were on the same face of the molecule while one $CH_3$ group (δ 0.86) and a methine proton resonance (δ 0.97-1.00) were on the opposite face. These data confirmed the relative configuration of the asymmetric centres in the decaline moiety of (1).

The structure of aliphatic portion in testacein (1), followed from a comparison of $^{13}$C-NMR data of (1) with those of the known diterpene aldehydes (6) and (7) (Figure 4). As the reported $^{13}$C-NMR signals for (6) and (7) were simply listed rather than assigned, the data for these three compounds are listed in increasing order of chemical shifts for comparison (Table 3) [Kinoshita *et al.* 2003; Hua *et al.* 2004; Margaros *et al.* 2006; George *et al.* 2010]. Most of the signals are well matched except at and in the vicinity of C8 (at C7, C8, C9, C11 and C12), which is not surprising since the 8-OH group in (6) is replaced by an 8-*O*-substituted benzyl group in (1). Thus the most significant variations were observed at C8 [δ 78.5 in (1) and δ 72.9 in (6) and at C12 [δ 21.0 in (1) and 25.4 in (6)]. However, in pestalotiopene C (3) the chemical shift of C8 was recorded at δ 78.5, consistent with that observed in testacein (1) (Hammerschmidt *et al.* 2014). Further, the observed correlations in gHMBC spectrum of (1) (Figure 2) were consistent with the proposed structure of the aliphatic portion of this molecule.

The specific rotation of testacein (1) was measured as $[\alpha]_D^{24} = -8.6$ (c 0.12, $(CD_3)_2CO$), while specific rotation of the terpene (7) has been variously measured as $[\alpha]_D^{21} = +37.0$ (c 1.0, $CDCl_3$), $[\alpha]_D^{22} = +39.2$ (c 0.65, $CDCl_3$) and $[\alpha]_D^{22} = +31.9$ (c 0.0075, $CDCl_3$), while the specific rotation of the diastereomeric aldehyde (6) was recorded as $[\alpha]_D^{27} = -38.2$ (c= 0.84, $CDCl_3$) [Kinoshita *et al.* 2003; Hua *et al.* 2004; George *et al.* 2010]. As the absolute configuration of (6) is known with confidence, the absolute configuration of the aliphatic portion of testacein (1) was tentatively assigned as (5*R*,8*S*,9*S*,10*R*) as shown in Figure 4 (while recognized that alternative configurations such as (5*S*,8*R*,9*R*,10*S*) as observed in the fungal pestalotiopenes would also be possible).

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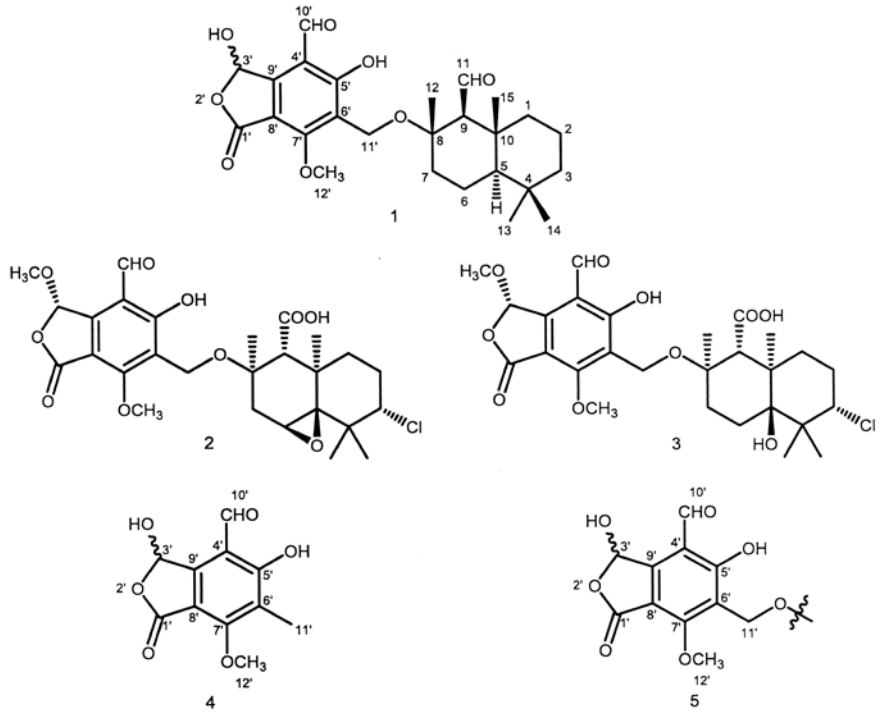


Figure 1. Structure of testacein (1), pestalotiopene A (2), pestalotiopene C (3), cyclopaldic acid (4) and substructure (5).



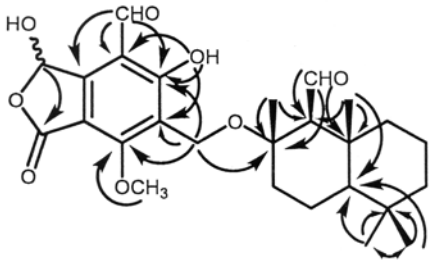

Figure 2. Correlations in gHMBC spectrum of testacein (1)

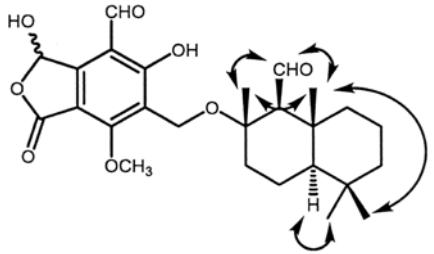

Figure 3. Correlations in NOESY spectrum of testacein (1)

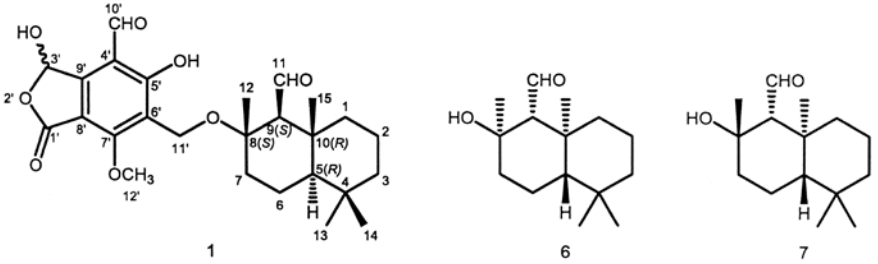


Figure 4. Structures of testacein (1) and terpenes (6) and (7)

Table 1. $^{1}$H-NMR data for compounds (1), (2), (3) and (4) (δ ppm)

| | 1 $(CD_3)_2CO$ | 1 $(CDCl_3)$ | 2 $(CDCl_3)^{\$}$ | 2 $(CD_3)_2SO$* | 3 $(CD_3)_2SO$* | 4 $(CDCl_3)^{\#}$ |
|---|---|---|---|---|---|---|
| 1 | 1.14, 1.67 | 1.13, 1.67 | 1.44, 2.41 | 1.60 | 1.22 | -- |
| 2 | 1.39, 1.62-1.66 | 1.44, 1.63 | 2.04-2.07, 2.17 | 1.90, 2.15 | 1.92, 2.07 | -- |
| 3 | 1.21-1.24, 1.38-1.41 | 1.18, 1.40 | 3.94 | 4.01 | 4.51 | -- |
| 5 | 0.97-1.00 | 0.93 | -- | -- | -- | -- |
| 6 | 1.46-1.49, 1.79-1.83 | 1.39, 1.79 | 3.24 | 3.36 | 1.97, 2.05 | -- |
| 7 | 1.77, 2.12 | 1.70, 2.11 | 2.09, 2.84 | 2.05, 2.79 | 1.83, 2.28 | -- |
| 9 | 2.20 | 2.20 | 3.25 | 3.25 | 3.31 | -- |
| 11 | 9.93-9.94 | 9.94 | -- | -- | -- | -- |
| 12 | 1.63 | 1.63 | 1.40 | 1.36 | 1.39 | -- |
| 13 | 0.86 | 0.84 | 0.95 (1.22) | 0.95 (1.25) | 1.09 (1.15) | -- |
| 14 | 0.91 | 0.90 | 1.22 (0.95) | 1.25 (0.95) | 1.15 (1.09) | -- |
| 15 | 1.19 | 1.20 | 1.47 | 1.50 | 1.61 | -- |
| 3′ | 7.02 | 6.67 | 6.44 | 6.76 | 6.75 | 6.80 |
| 3′-OH | 7.39 | 5.64 | -- | -- | -- | -- |
| 5′-OH | 12.36 | 12.02 | 12.42 | -- | -- | 12.29 |
| 10′ | 10.30 | 10.16 | 10.08 | 10.22 | 10.20 | 10.21 |
| 11′ | 4.55 | 4.54 | 4.59, 4.71 | 4.57, 4.75 | 4.58, 4.61 | 2.16 |
| 12′ | 4.17 | 4.18 | 4.34 | 4.19 | 4.16 | 4.20 |

$ Hemberger *et al.*, * Hammerschmidt *et al.*, # Achenbach *et al.*)

Table 2. $^{13}$C-NMR data for compounds (1), (2), (3) and (4) (δ ppm)

| | 1 $(CD_3)_2CO$ | 1 $(CDCl_3)$ | 2 $(CDCl_3)^{\$}$ | 2 $(CD_3)_2SO$* | 3 $(CD_3)_2SO$* | 4 $(CD_3)_2CO^{\#}$ |
|---|---|---|---|---|---|---|
| 1 | 40.6 | 40.2 | 35.5 | 34.9 | -- | -- |
| 2 | 18.8 | 18.2 | 28.9 | 28.9 | -- | -- |
| 3 | 42.5 | 41.8 | 68.4 | 68.4 | 71.0 | -- |
| 4 | 33.8 | 33.4 | 41.2 | 41.2 | 46.2 | -- |
| 5 | 56.0 | 55.5 | 66.8 | 66.0 | 78.5 | -- |
| 6 | 20.7 | 20.1 | 53.5 | 53.7 | 39.0 | -- |
| 7 | 39.5 | 39.0 | 32.9 | 33.6 | 34.6 | -- |
| 8 | 78.5 | 78.7 | 75.7 | 74.6 | 78.5 | -- |
| 9 | 70.2 | 69.6 | 55.2 | 52.9 | 56.4 | -- |
| 10 | 39.3 | 38.8 | 36.6 | 36.0 | -- | -- |
| 11 | 206.2 | 207.0 | 171.0 | 173.1 | -- | -- |
| 12 | 21.2 | 21.0 | 26.9 | 27.5 | 28.7 | -- |
| 13 | 21.8 | 21.7 | 22.6 (21.6) | 21.1 (22.6) | 18.5 (25.1) | -- |
| 14 | 33.8 | 33.5 | 21.6 (22.6) | 22.6 (21.1) | 25.1 (18.5) | -- |
| 15 | 17.4 | 17.4 | 18.3 | 17.8 | 17.6 | -- |
| 1′ | 165.5 | 165.2 | 164.5 | 164.7 | -- | 167.5 |
| 3′ | 96.0 | 94.8 | 99.9 | 100.8 | -- | 96.0 |
| 4′ | 112.7 | 112.2 | 111.6 | 113.1 | -- | 112.3 |
| 5′ | 167.9 | 167.8 | 167.0 | 167.0 | -- | 166.0 |
| 6′ | 122.6 | 121.0 | 119.6 | 122.1 | -- | 121.5 |
| 7′ | 164.8 | 164.0 | 163.9 | 162.8 | -- | 163.4 |
| 8′ | 111.2 | 109.7 | 109.5 | 111.5 | -- | 111.0 |
| 9′ | 155.6 | 153.8 | 152.4 | 151.3 | -- | 153.7 |
| 10′ | 194.4 | 192.1 | 191.8 | 195.2 | -- | 194.7 |
| 11′ | 51.1 | 51.6 | 52.2 | 51.7 | -- | 8.1 |
| 12′ | 64.0 | 64.0 | 64.1 | 63.3 | 62.7 | 63.1 |



Table 3. Comparison of $^{13}$C-NMR data for compounds (1), (6) and (7)

| | 1 $(CD_3)_2CO$ | 1 $(CDCl_3)$ | 6 $(CDCl_3)^{\alpha}$ | 6 $(CDCl_3)^{\beta}$ | 6 $(CDCl_3)^{\gamma}$ | 6 $(CDCl_3)^{\delta}$ | 7 $(CDCl_3)^{\beta}$ |
|---|---|---|---|---|---|---|---|
| 1 | 17.4 | 17.4 | 17.4 | 17.6 | 17.7 | 17.7 | 16.9 |
| 2 | 18.8 | 18.2 | 18.1 | 18.2 | 18.3 | 18.3 | 17.9 |
| 3 | 20.7 | 20.1 | 19.8 | 19.9 | 20.0 | 20.0 | 18.2 |
| 4 | 21.2 | 21.0 | 21.3 | 21.4 | 21.5 | 21.5 | 21.5 |
| 5 | 21.8 | 21.7 | 25.2 | 25.3 | 25.4 | 25.4 | 31.0 |
| 6 | 33.8 | 33.4 | 33.1 | 33.2 | 33.3 | 30.5 | 33.3 |
| 7 | 33.8 | 33.5 | 33.2 | 33.3 | 33.4 | 33.5 | 33.5 |
| 8 | 39.3 | 38.8 | 37.3 | 37.4 | 37.4 | 37.5 | 39.8 |
| 9 | 39.5 | 39.0 | 39.7 | 39.8 | 39.8 | 39.9 | 40.2 |
| 10 | 40.6 | 40.2 | 41.5 | 41.6 | 41.7 | 41.8 | 41.4 |
| 11 | 42.5 | 41.8 | 42.7 | 42.7 | 42.7 | 42.9 | 41.6 |
| 12 | 56.0 | 55.5 | 55.0 | 55.1 | 55.2 | 55.3 | 55.1 |
| 13 | 70.2 | 69.6 | 71.2 | 71.3 | 71.3 | 71.4 | 69.3 |
| 14 | 78.5 | 78.7 | 72.7 | 72.8 | 72.7 | 72.9 | 71.3 |
| 15 | 206.2 | 207.0 | 208.0 | 208.2 | 207.7 | 208.3 | 209.7 |

α Margaros *et al.*, β George *et al.*, γ Kinoshita *et al.*, δ Hua *et al.*