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THE CHEMISTRY OF *NOTHEIA ANOMALA*, III: TWO NEW METHYLENE-INTERRUPTED TRISEPOXYLIPIDS<sup>1</sup>

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**ABSTRACT.**—Two new methylene-interrupted trisepoxy lipids, **3** and **4**, have been isolated from the Australian epiphytic marine brown alga *Notheia anomala*. Structures were assigned on the basis of detailed spectroscopic analysis.

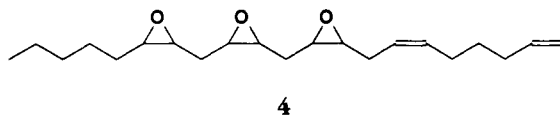
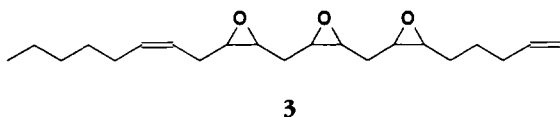
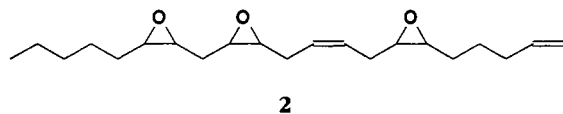
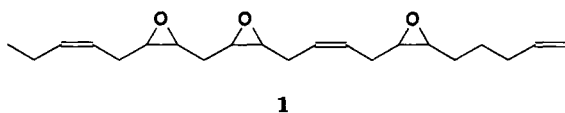
As part of our ongoing investigations into the chemistry of southern Australian marine organisms, we have reported (1,2) a series of unusual oxygenated lipids from the epiphytic marine brown alga *Notheia anomala* Harvey and Bailey (Notheiaceae; Fucales). This brown alga is found as an epiphyte on the abundant brown alga *Hormosira banksii*, both of which are unique to the coasts of Australia and New Zealand. In our most recent report on the chemistry of *N. anomala* (2), we included a description of the isolation and structure elucidation of two novel C<sub>21</sub> trisepoxy lipids **1** and **2**. Subsequent investigations into the extracts that yielded these metabolites have resulted in the isolation and identification of two further trisepoxy lipids **3** and **4**. These latter compounds, which are present as very minor components in *N. anomala*, form the subject of this report.

During an earlier investigation of a mixed fraction containing both trisepoxy lipids **1** and **2** (2) a third very minor compound was detected. Although at that time this minor component eluded isolation, spectroscopic data obtained on mixed fractions suggested that it too may be a trisepoxy lipid. A careful reinvestigation of this material, making use of C<sub>18</sub> reversed-phase hplc, has resulted in the isolation of a new C<sub>21</sub> trisepoxy lipid **4**. Likewise, examination of a more polar CH<sub>2</sub>Cl<sub>2</sub> soluble fraction from the same

earlier investigation (2) yielded another very minor new C<sub>21</sub> trisepoxy lipid **3**.

The oils of the moderately stable colorless oil **3** did not display a molecular ion peak; however, an [M + H]<sup>+</sup> ion peak was observed at *m/z* 335 in both the FABMS (thioglycerol) and CIMS (isobutane). This, together with <sup>1</sup>H- and <sup>13</sup>C-nmr data, was interpreted as supporting a molecular formula of C<sub>21</sub>H<sub>34</sub>O<sub>3</sub> requiring five double bond equivalents. Instrumental limitation precluded measurement of accurate masses for the [M + H]<sup>+</sup> ions for both **3** and **4**, and regrettably prevented an independent confirmation of their molecular formula. Attempts to obtain combustion analysis data were complicated by decomposition during handling, together with a propensity for these oils to trap solvent, and by the small amount of material available. Further analysis of the nmr data permitted two of the double bond equivalents to be attributed to a monosubstituted double bond [ $\delta$  4.92 (ddt, *J* = 10.3, 3.0, 1.2 Hz), 4.99 (ddt, *J* = 17.1, 3.0, 3.7 Hz), 5.82 (ddt, *J* = 17.1, 10.3, 6.8 Hz), 115.0 (t), 138.1 ppm (d)] and a 1,2-disubstituted double bond [ $\delta$  5.43 (m), 5.55 (m), 123.2 (d), 133.1 ppm (d)]. Comparison of the <sup>13</sup>C-nmr chemical shifts attributed to the methylene carbons allylic to the 1,2-disubstituted double bond (26.3, 27.4 ppm) with model compounds (2) suggested a *Z* geometry. The remaining three double bond equivalents were attributed to three 1,2-disubstituted epoxide functionalities, as identified by the presence of six epoxymethine carbon resonances [54.0 (d), 54.1 (d),

<sup>1</sup>For Part II, see Murray *et al.* (2).



54.1 (d), 54.2 (d), 56.3 (d), and 56.5 ppm (d)] and an epoxymethine proton resonance ( $\delta$  3.00–3.15) integrating for six protons. The nmr data also revealed a primary methyl moiety [ $\delta$  0.89 (bt,  $J = 6.9$  Hz); 14.0 ppm (q)]. Thus **3** was an acyclic hydrocarbon incorporating a terminal methyl group and a terminal double bond, as well as a *cis* 1,2-disubstituted double bond and three 1,2-disubstituted epoxide moieties. Furthermore, as the methyl resonance in the  $^1\text{H}$ -nmr spectrum of **3** was broad at ca. 0.9, rather than sharp at ca. 1.0, **3** did not incorporate a  $\Delta^{3,4}$  double bond (1,2).

A 2D-nmr COSY experiment on **3** confirmed correlations from the epoxymethine multiplet ( $\delta$  3.00–3.15) via a deshielded methylene ( $\delta$  2.21 and 2.37) to two olefinic methine multiplets ( $\delta$  5.43 and 5.55) associated with the 1,2-disubstituted double bond. Furthermore, these olefinic methines displayed correlations via a single allylic methylene ( $\delta$  2.03) to an aliphatic methylene envelope ( $\delta$  1.37). These correlations could be attributed to the sequence C-10 to C-

4 as shown on the structure diagram. A second sequence of correlations could be traced from the terminal double bond protons via an allylic methylene multiplet ( $\delta$  2.13) to two anisotropic methylene proton resonances ( $\delta$  1.53 and 1.68). These resonances in turn displayed correlations via two anisotropic methylene proton resonances ( $\delta$  1.56 and 1.83) to the epoxymethine multiplet ( $\delta$  3.00–3.15). These correlations could be attributed to the sequence C-21 to C-15 as shown on the structure diagram. The absence of additional 2D nmr COSY correlations to the epoxymethine proton resonance ( $\delta$  3.00–3.15) together with the  $^{13}\text{C}$ -nmr chemical shifts for C-10 through to C-15 supported the structure for **3** as shown. In particular, identical  $^{13}\text{C}$ -nmr chemical shifts for C-12 and C-13, and very similar chemical shifts for C-11 and C-14, and C-10 and C-15, respectively, suggested that these carbons were in very similar environments. Compound **3** represents a methylene-interrupted trisepoxylipid isomer of the previously reported trisepoxylipid **2**.

The stereochemistry about the chiral centers remains undetermined.

Detailed analysis of the  $^1\text{H}$ -nmr,  $^{13}\text{C}$ -nmr and ms spectral data for **4** confirmed not only that it was isomeric with **2** and **3**, but that it also incorporated a terminal primary methyl and a terminal mono-substituted double bond, along with a cis 1,2-disubstituted double bond and three 1,2-disubstituted epoxide moieties. Correlations in the 2D nmr COSY spectrum could be seen from the epoxy-methine multiplet ( $\delta$  3.00–3.16) via an allylic methylene ( $\delta$  2.22 and 2.38) to two olefinic methine multiplets ( $\delta$  5.44 and 5.55) associated with the 1,2-disubstituted double bond. Furthermore, these same olefinic methine protons displayed correlations via an allylic methylene ( $\delta$  2.08) to an aliphatic methylene ( $\delta$  1.49) that was in turn further correlated via an allylic methylene ( $\delta$  2.08) to the monosubstituted double bond protons. This sequence of correlations was consistent with the partial structure C-12 to C-21 as can be seen on the structure diagram. As with **3**, consideration of the  $^{13}\text{C}$  nmr for C-6 to C-13 confirmed that the three epoxide moieties were methylene-interrupted and that the complete structure for **4** must be as shown. Once again the stereochemistry about the chiral centers remains undetermined.

As with all the *N. anomala* metabolites previously reported (1,2) the biosynthesis of the trisepoxylipids **3** and **4** would appear to involve decarboxylation of a  $\text{C}_{22}$  unsaturated fatty acid followed by enzyme-mediated epoxidation. At this stage the biological role of these unusual oxygenated lipids remains unknown.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—Procedures were as previously reported (1). Extracts obtained from an earlier investigation of *N. anomala* (2) were re-examined as outlined below.

**FRACTION 1.**—A fraction previously known to contain the two trisepoxylipids **1** and **2** was subjected to careful reversed-phase hplc (injec-

tions of ca. 1 mg of mixture onto a Phenomenex 5  $\mu$   $\text{C}_{18}$  250  $\times$  10 mm hplc column, eluted with 5%  $\text{H}_2\text{O}/\text{MeOH}$  at 2.0 ml/min). Taking advantage of peak shaving plus repeated re-injection of partially purified fractions, it was possible to isolate a small amount of the pure compound **4** (19 mg, 0.0020% dry wt).

**FRACTION 2.**—Similar careful examination of a more polar fraction yielded, after mpls [10% stepwise elution from petroleum ether (40–60°) to EtOAc through silica] followed by normal phase hplc (injection of ca. 1–3 mg onto a Phenomenex 5  $\mu$  silica 250  $\times$  10 mm hplc column, elution with 10% EtOAc/hexane at 2.0 ml/min), a small amount of the pure compound **3**. Once again purification was assisted by peak shaving and re-injections.

(6Z)-9,10:12,13:15,16-Trisepoxybenicosa-6-20-diene [**3**].—A moderately stable colorless oil:  $[\alpha]_{\text{D}}^{20} +35.5^\circ$  ( $c = 0.1$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  nmr (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.89 (bt, 6.9 Hz,  $\text{H}_3$ -1), 1.2–2.4 (bm, methylene envelope), 3.0–3.15 (bm, H-9, -10, -12, -13, -15, -16), 4.92 (ddt, 10.3, 3.0, 1.2 Hz,  $\text{H}_{\text{cis}}$ -21), 4.99 (ddt, 17.1, 3.0, 3.7 Hz,  $\text{H}_{\text{trans}}$ -21), 5.43 (m, H-7), 5.55 (m, H-6), 5.82 (ddt, 17.1, 10.3, 6.8 Hz, H-20);  $^{13}\text{C}$  nmr (100 MHz,  $\text{CDCl}_3$ ) (assignments with the same superscript can be interchanged) 14.0 (C-1), 22.5 (C-2), 25.8 (C-18), 26.3 (C-8), 27.0 (C-11<sup>a</sup>), 27.3 (C-14<sup>a</sup>), 27.3 (C-17<sup>a</sup>), 27.4 (C-5), 29.2 (C-4), 31.5 (C-3), 33.4 (C-19), 54.0 (C-10<sup>b</sup>), 54.1 (C-12<sup>b</sup>), 54.1 (C-13<sup>b</sup>), 54.2 (C-15<sup>b</sup>), 56.3 (C-9<sup>c</sup>), 56.5 (C-16<sup>c</sup>), 115.0 (C-21), 123.2 (C-7), 133.1 (C-6), 138.1 ppm (C-20); fabms (thioglycerol)  $m/z$   $[\text{M} + \text{H}]^+$  335; cims (isobutane)  $[\text{M} + \text{H}]^+$  335.

(15Z)-6,7:9,10:12,13-trisepoxybenicosa-15-20-diene [**4**].—A moderately stable colorless oil:  $[\alpha]_{\text{D}}^{20} +33.3^\circ$  ( $c = 0.8$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  nmr (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.89 (bt, 6.8 Hz,  $\text{H}_3$ -1), 1.2–2.4 (bm, methylene envelope), 3.0–3.16 (bm, H-6, -7, -9, -10, -12, -13), 4.92 (ddt, 10.3, 3.0, 1.2 Hz,  $\text{H}_{\text{cis}}$ -21), 4.99 (ddt, 17.1, 3.0, 3.7 Hz,  $\text{H}_{\text{trans}}$ -21), 5.44 (m, H-15), 5.55 (m, H-16), 5.80 (ddt, 17.1, 10.3, 6.8 Hz, H-20);  $^{13}\text{C}$  nmr (100 MHz,  $\text{CDCl}_3$ ) (assignments with the same superscript can be interchanged) 14.0 (C-1), 22.6 (C-2), 26.3 (C-5<sup>a</sup>), 26.4 (C-18<sup>a</sup>), 26.8 (C-14<sup>a</sup>), 27.0 (C-8<sup>a</sup>), 27.2 (C-4<sup>a</sup>), 27.8 (C-11<sup>a</sup>), 28.7 (C-17<sup>a</sup>), 31.7 (C-3), 33.2 (C-19), 54.0 (C-9<sup>b</sup>), 54.0 (C-12<sup>b</sup>), 54.1 (C-10<sup>b</sup>), 54.2 (C-7<sup>b</sup>), 56.3 (C-13<sup>c</sup>), 56.5 (C-6<sup>c</sup>), 114.6 (C-21), 123.9 (C-15), 132.4 (C-16), 138.5 ppm (C-20); fabms (thioglycerol)  $m/z$   $[\text{M} + \text{H}]^+$  335; cims (isobutane)  $[\text{M} + \text{H}]^+$  335.

## ACKNOWLEDGMENTS

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