Chemical Constituents from the Colombian Medicinal Plant *Niphogeton ternata*

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Two coumarins and one polyacetylene, 5-*O*-(3-chloro-2-hydroxy-3-methylbutyl)-8-methoxypsoralen (1), 2′,3′-dihydro-jatamansin (2), and 10-chloro-1-heptadecene-4,6-diyne-3,8,9-triol (3), along with 15 known compounds (4—18), were isolated from the methanol extract of *Niphogeton ternata.* Their structures were elucidated by spectroscopy.

Key words *Niphogeton ternata*; Umbelliferae; coumarin; polyacetylene

Niphogeton ternata WILLD. ex SCHLTR. (Umbelliferae), a rare folk medicinal herb grown in Colombia, has been used for the treatment of dysentery, colitis, and rheumatism.[1)] Recently, volatile components from a plant of the same genus, *Apium graveolens* var. *filicinum*, was found to have cytotoxic effects on human cancer cell lines.[2)] In our search for pharmacologically active compounds from crude drugs of plant origin,[3)] we have begun to study the chemical constituents of *Niphogeton ternata.* This paper deals with the isolation and structural elucidation of three new and 15 known compounds from the ethyl acetate-soluble fraction of methanol extracts of *N. ternata.* The ethyl acetate-soluble fraction from *N. ternata* was separated by repeated silica gel column chromatography, HPLC, and gel-permeation chromatography (GPC), to give compounds **1**—**18**.

Compound **1** was obtained as an amorphous solid and its high resolution electron impact-mass spectrum (HR-EI-MS) showed a molecular ion peak at *m/z* 352.0705, indicating a molecular formula of $C_{17}H_{17}ClO_6$. The IR spectrum showed hydroxyl and carbonyl bands (3429, 1728 cm^{-1}), and its UV absorption spectrum showed the absorptions of a furanocoumarin derivative (306, 266, 249, 220 nm).[4)] The ^{1}H-NMR spectral data of **1** revealed four aromatic protons [δ_H 8.17, 6.30 (each 1H, d, J=9.9 Hz); 7.64, 6.99 (each 1H, d, J=2.2 Hz)], one oxygenated methine [δ_H 4.07 (1H, dd, J=7.8, 2.8 Hz)], and one oxygenated methylene [δ_H 4.60 (1H, dd, J=10.0, 2.8 Hz), 4.33 (1H, dd, J=10.0, 7.8 Hz)], as well as one methoxy and two tertiary methyl groups. Its ^{13}C-NMR spectrum showed 11 carbon signals in the lower field region, consisting of four aromatic methines, six quaternary carbons, and one carboxyl carbon signal. This suggested the presence of a furanocoumarin framework. In addition, one oxygenated methylene, one oxygenated methine, one methoxy, and two methyl groups were also observed. Compound **1** was assumed to be a linear furanocoumarin derivative, and its ^{13}C-NMR spectrum was similar to that of saxalin (**7**),[4)] except for C-7, -8, -8a and one methoxy group (Fig. 1).

In the heteronuclear multiple bond connectivity (HMBC) spectrum, the proton signal at δ_H 6.99 (H-3′) correlated with the carbon signals at δ_C 145.6 (C-2′), 149.9 (C-7), and 142.9 (C-5), the signal at δ_H 7.64 (H-2′) correlated with the signals at δ_C 116.3 (C-6) and 149.9 (C-7), and the signal at δ_H 8.17 (H-4) correlated with the signals at δ_C 142.9 (C-5), 144.5 (C-8a), and 160.4 (C-2), while the signal at δ_H 6.30 (H-3) correlated with the signals at δ_C 108.7 (C-4a) and 160.4 (C-2). In the nuclear Overhauser effect spectroscopy (NOESY) spectrum, the proton signal at δ_H 6.99 (H-3′) correlated with the signals at δ_H 4.33 (H-3b″) and 7.64 (H-2′), and the proton signal at δ_H 8.17 (H-4) correlated with 6.30 (H-3) and 4.60 (H-3a″). These observations confirmed the presence of a furanocoumarin skeleton. Moreover, the methylene proton signal (δ_H 4.33, H-3b″) of the side chain [3(or 2)-chloro-2(or 3)-hydroxy-3-methylbutyl, based on an investigation of ^{1}H–^{1}H correlation spectroscopy (COSY) and HMBC spectra] correlated with the signal at δ_C 142.9 (C-5), and the methoxy proton signal at δ_H 4.18 correlated with the signal at δ_C 129.3 (C-8). Thus the side chain and methoxy group were assigned positions at C-5 and C-8, respectively. Acetylation of **1** (Ac_2O/pyridine) gave a monoacetate **1a**, which indicated the presence of a secondary hydroxyl group in the side chain. Thus the side chain was proposed to be 3-chloro-2-hydroxy-3-methylbutyl. Therefore the structure of **1** was determined as shown in Fig. 1.

Compound **2** had a molecular formula of $C_{19}H_{22}O_5$ based on HR-FAB-MS. Its ^{1}H-NMR spectral data revealed four aromatic protons [δ_H 7.63, 6.24 (each 1H, d, J=9.4 Hz); 7.26, 6.79 (each 1H, d, J=8.6 Hz)], one oxygenated methine [δ_H 5.13 (1H, t, J=5.3 Hz)], one terminal methyl [δ_H 0.87 (3H, t, J=7.5 Hz)], one secondary methyl [δ_H 1.14 (3H, d, J=7.0 Hz)], and two tertiary methyls [δ_H 1.36 (6H, s)]. The ^{13}C-NMR spectrum showed nine carbons in the lower field region, indicating the presence of a coumarin skeleton. In addition, four methyl, two methylene, two methine, one oxygenated quaternary carbon, and one carboxylic carbon signals were also observed. The ^{13}C-NMR spectrum of **2** was similar to that of **14**, which was identified as jatamansin.[5)] Thus **2** was a coumarin derivative, and two sets of coupled aromatic protons indicated that the side chain was bonded to the coumarin skeleton at C-7 and C-8. From the ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectra, the partial structures –CH_2CH(–O–)– and $CH_3CH_2CH(CH_3)$– were obtained. In the HMBC spectrum, the proton signals at δ_H 1.36 (H_3-12, -13) correlated with the signals at 76.8 (C-11) and 69.3 (C-10), the signal at δ_H 5.13 (H-10) correlated with the signals at δ_C 22.5, 25.5

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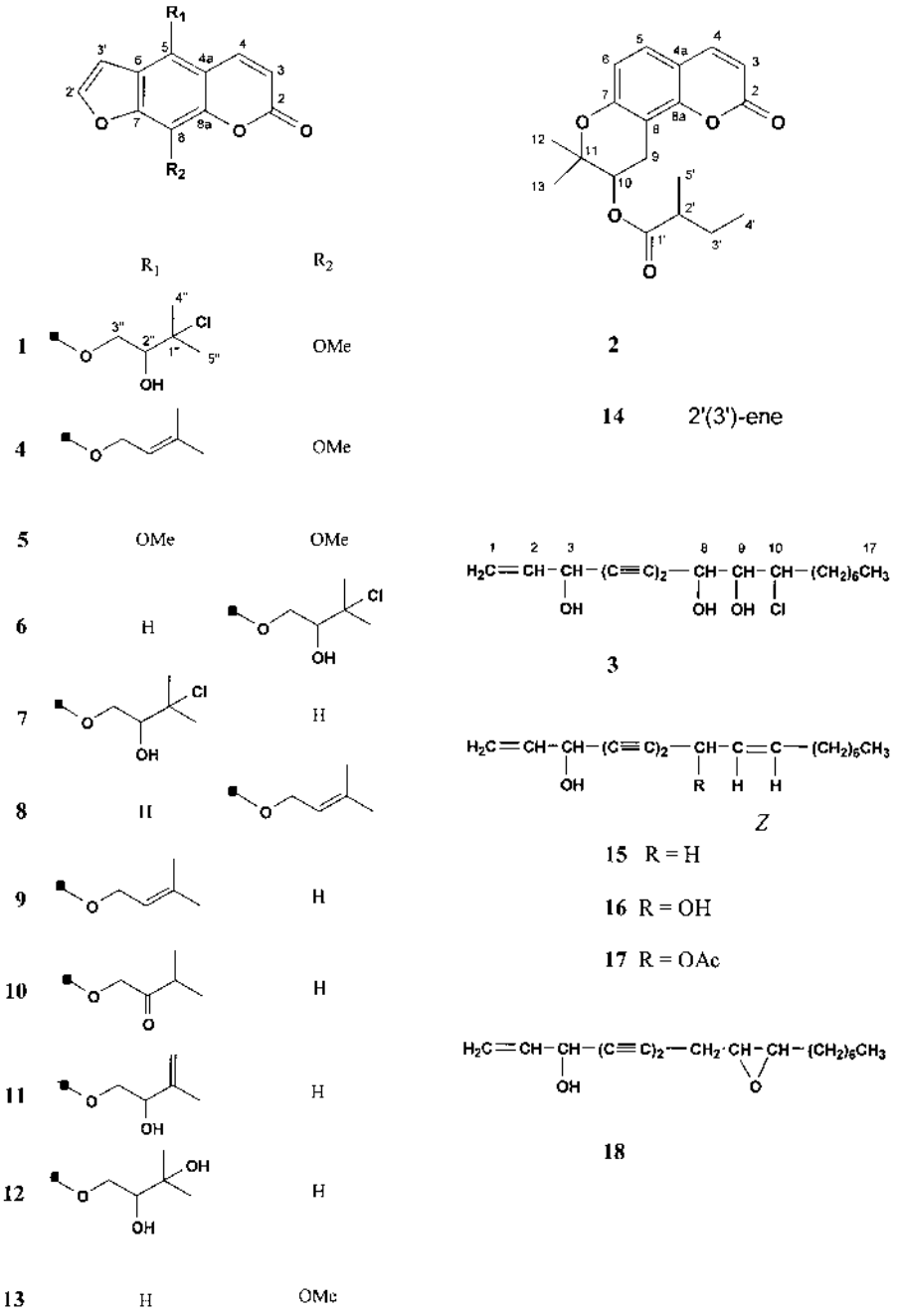

Fig. 1

(C-12, -13), 175.9 (C-1′), and 107.3 (C-8), and the signal at δ_H 3.22 (H-9a) with the signals at δ_C 156.4 (C-7), 107.3 (C-8), and 153.4 (C-8a), while the methyl proton signal at δ_H 1.14 (H_3-5′) correlated with the signals at δ_C 26.7 (C-3′), 41.1 (C-2′), and 175.9 (C-1′). Thus the partial structure of 1,1-dimethyl-1,2-dioxgenated propyl was directly bonded to the coumarin skeleton at C-8, and the 2′,3′-tigloyl was bonded to C-10 by an ester bond. Furthermore, there was another ring in addition to the coumarin skeleton, accounting for the degrees of unsaturation of **2** indicated. Thus the remaining bonded positions C-7 and C-11 were linked by an ether bond. Therefore the structure of **2** was determined as shown in Fig. 1.

Compound **3** was assigned the molecular formula $C_{17}H_{25}ClO_3$ based on HR-FAB-MS. Its IR spectrum showed hydroxyl and acetylene absorption bands at 3413 and 2362 cm^{-1}. The ^{1}H-NMR spectral data of **3** revealed a terminal vinyl group [δ_H 5.95 (1H, ddd, J=17.0, 10.2, 5.2 Hz), 5.49 (1H, d, J=17.0 Hz), and 5.28 (1H, d, J=10.2 Hz)], three oxygenated methines [δ_H 4.96 (1H, d, J=5.2 Hz), 4.55 (1H, d, J=6.3 Hz), 3.74 (1H, dd, J=6.3, 4.2 Hz)], and one methyl group [δ_H 0.90 (3H, t, J=7.0 Hz)]. The ^{13}C-NMR spectral data showed 17 carbons: a terminal methylene, five methines, six methylenes, and one methyl group. In addition, there were four carbon signals (δ_C 78.4, 77.7, 70.7, 70.0) due to *sp* carbons which could be assigned to the diacetylene moiety based on a consideration of the molecular formula. The chemical shifts and coupling patterns of H-3 (4.96, d, 5.2) and H-8 (4.55, d, 6.3) suggested that the diacetylene moiety could be inserted between C-3 and C-8. In the HMBC spec-

trum, the proton signal at δ_H 4.96 (H-3) correlated with the carbon signals at δ_C 117.6 (H-1), 78.4 (C-4), and 70.7 (C-5), the signal at δ_H 4.55 (H-8) correlated with the signals at δ_C 70.0 (C-6), 77.7 (C-7), and 64.4 (C-10), and the signal at δ_H 3.74 (H-9) correlated with the signals at δ_C 77.7 (C-7) and 34.6 (C-11). Moreover, acetylation of **3** gave triacetate **3a**, and H-3, H-8, and H-9 showed significant downfield chemical shifts compared with those of **3**. Thus the structure of **3** was determined to be 10-chloro-1-heptadecene-4,6-diyne-3,8,9-triol.

By comparing spectral data, the known compounds **4**—**18** were identified as follows: cnidilin (**4**),[6] isopimpinellin (**5**),[7] 8-(3-chloro-2-hydroxy-3-methylbutoxy)psoralen (**6**),[8] saxalin (**7**),[4] imperatorin (**8**),[7] isoimperatorin (**9**),[4] isooxypeucedanin (**10**),[4] pabulenol (**11**),[9] oxypeucedanin hydroate (**12**),[4] xanthotoxin (**13**),[8] jatamansin (**14**),[5] falcarinol (**15**),[10] falcarindiol (**16**),[10] 8-acetoxyfalcarinol (**17**),[11] and panaxydol (**18**).[12]

Experimental

General Experimental Procedures NMR experiments were carried out on a Bruker ARX-400 instrument: ^{1}H-NMR, 400 MHz; and ^{13}C-NMR, 100 MHz, both with tetramethylsilane as an internal standard. MS data were obtained on a JEOL JMSD-300 instrument. The chromatography columns were a Silica-gel 60 (Merck) and Toyopearl HW-40 (Pharmacia); HPLC was performed on a JASCO Gulliver Series PU-986/987 (pump), and RI930 and UV970 (detectors). The column types were GPC (Asahipak, GS-310 2G, MeOH) and silica gel HPLC (YMC-Pack SIL-06 SH-043-5-06, 250×20 mm); IR spectra were recorded on a 1720 Infrared Fourier Transform spectrometer (Perkin-Elmer); UV spectra were obtained on a UV 2100 UV-VIS recording spectrometer (Shimadzu). Optical rotation was measured with a JASCO DIP-370 digital polarimeter.

Plant Material The aerial part of *N. ternata* WILLD. ex SCHLTR. was collected in August 2000 from Bogota, Colombia, and identified by Dr. Cristina Garzon. A voucher specimen (CQ-00JC004 NV) was deposited at the Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Colombia.

Extraction and Isolation The aerial part (2.2 kg) of *N. ternata* was crushed and extracted 3× with MeOH (20 l each) at 60 °C for 6 h. The MeOH extracts were concentrated *in vacuo* to give a residue (230 g), which was partitioned between EtOAc and H_2O. The EtOAc layer was concentrated to give a residue (67 g), which was chromatographed on a silica gel (800 g) column (80×850 mm). The column was eluted with solvents of increasing polarity [$CHCl_3$–MeOH (99 : 1, 95 : 5, 9 : 1, MeOH)] to give 20 fractions (fractions 1—20). Combined fractions 6 and 7 (5.7 g) were chromatographed on a Toyopearl HW-40 ($CHCl_3$–MeOH, 2 : 1) column to give four fractions (fractions 6.1—6.4). Fraction 6.3 was crystallized using $CHCl_3$–MeOH to give **8** (550 mg), and the mother liquid was then subjected to Si HPLC (hexane–EtOAc, 3 : 1) to give **4** (54 mg), **9** (69 mg), and **15** (9 mg). Fraction 9 (7 g) was chromatographed over Toyopearl HW-40 ($CHCl_3$–MeOH, 2 : 1) to give four fractions (fractions 9.1—9.4). Fraction 9.2 (yield 0.6 g of 2.5 g) was separated by GPC and Si HPLC (hexane–EtOAc, 5 : 2) to give **2** (7 mg). Fraction 9.3 (yield 0.5 g of 2.0 g) was separated by Si HPLC (hexane–EtOAc, 2 : 1) to give **14** (85 mg). Fraction 10 (5.9 g) was chromatographed on Toyopearl HW-40 to give four fractions (fractions 10.1—10.4). Fraction 10.3 (yield 0.5 g of 2.3 g) was separated by Si HPLC (hexane–EtOAc, 2 : 1) to give **1** (8 mg), **10** (10 mg), **18** (7 mg), and two other fractions (fractions 10.3.1 and 10.3.2). Fraction 10.3.1 was further separated by preparative TLC (PTLC, $CHCl_3$–MeOH, 96 : 4) to give **6** (25 mg) and **11** (5 mg). Fraction 10.3.2 was separated by PTLC ($CHCl_3$–MeOH, 96 : 4) to give **5** (21 mg) and **17** (17 mg). Combined fractions 11 and 12 (yield 3 g of 6.7 g) were chromatographed on Toyopearl HW-40 to give four fractions (fractions 11.1—11.4). Fraction 11.3 was separated by Si HPLC to give **7** (57 mg), **13** (18 mg) and **16** (422 mg). Fraction 14 was chromatographed on Toyopearl HW-40 to give four fractions (fractions 14.1—14.4). Fraction 14.3 was separated by GPC and Si HPLC to give **3** (4 mg) and **12** (17 mg).

5-*O*-(3-chloro-2-hydroxy-3-methylbutyl)-8-methoxypsoralen (**1**): Amorphous powder, $[\alpha]_D^{25}$ +1.4° (c=0.8, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3429, 2925, 2856, 2360, 1728, 1593, 1473, 1348, 1207, 1147, 1063, 827. UV λ_{max} nm (MeOH) (log ε): 306 (3.95), 266 (4.08), 249 (4.12), 220 (4.29). ^{1}H-NMR ($CDCl_3$): δ: 8.17 (1H, d, J=9.9 Hz, H-4), 7.64 (1H, d, J=2.2 Hz, H-2′), 6.99 (1H, d, J=2.2 Hz, H-3′), 6.30 (1H, d, J=9.9 Hz, H-3), 4.60 (1H, dd, J=10.0, 2.8 Hz, H-3a″), 4.33 (1H, dd, J=10.0, 7.8 Hz, H-3b″), 4.07 (1H, dd, J=7.8, 2.8 Hz, H-2″), 4.18 (3H, s, –OMe), 1.69 (6H, s, H_3-4″, 5″). ^{13}C-NMR ($CDCl_3$): δ 160.4 (C-2), 149.9 (C-7), 145.6 (C-2′), 144.5 (C-8a), 142.9 (C-5), 139.4 (C-4), 129.3 (C-8), 116.3 (C-6), 113.4 (C-3), 108.7 (C-4a), 104.8 (C-3′), 77.5 (C-2″), 74.9 (C-3″), 71.4 (C-1″), 61.8 (–OMe), 29.2 (C-4″, 5″). EI-MS m/z (rel. int.): 352 $[M]^+$ (100), 316 (16), 259 (24), 231 (94), 217 (94), 189 (31), 160 (32), 147 (15), 104 (15), 77 (26), 57 (25), 43 (50). HR-EI-MS m/z: 352.0705. $C_{17}H_{17}ClO_6$ required 352.0714.

Acetylation of 1 Compound **1** (1.5 mg) was treated with Ac_2O (0.3 ml) and C_5D_5N (0.5 ml) at room temperature overnight. The reaction mixture was worked up in the usual way to give monoacetate **1a** (1 mg). Compound **1a**: ^{1}H-NMR ($CDCl_3$): δ: 8.06 (1H, d, J=9.8 Hz), 7.65 (1H, d, J=2.1 Hz), 6.97 (1H, d, J=2.1 Hz), 6.32 (1H, d, J=9.8 Hz), 5.51 (1H, dd, J=7.8, 2.7 Hz), 4.73 (1H, dd, J=10.2, 2.7 Hz), 4.54 (1H, dd, J=10.2, 7.8 Hz), 4.19 (3H, s), 2.16 (3H, s), 1.69 (3H, s), 1.64 (3H, s).

2′,3′-Dihydro-jatamansin (**2**): Amorphous powder, $[\alpha]_D^{25}$ +53.6° (c=0.8, MeOH). IR ν_{max}^{KBr} cm^{-1}: 2972, 2933, 1732, 1606, 1491, 1458, 1410, 1371, 1284, 1248, 1194, 1147, 1114, 1026, 835. UV λ_{max} nm (MeOH) (log ε) 324 (4.04), 255 (3.42), 245 (3.46), 218 (4.03). ^{1}H-NMR ($CDCl_3$): δ: 7.63 (1H, d, J=9.4 Hz, H-4), 7.26 (1H, d, J=8.6 Hz, H-5), 6.79 (1H, d, J=8.6 Hz, H-6), 6.24 (1H, d, J=9.4 Hz, H-3), 5.13 (1H, t, J=5.3 Hz, H-10), 3.22 (1H, dd, J=17.8, 5.3 Hz, H-9a), 2.92 (1H, dd, J=17.8, 5.3 Hz, H-9b), 2.38 (1H, m, H-2′), 1.66 (1H, m, H-3a′), 1.45 (1H, m, H-3b′), 1.36 (6H, s, H_3-12, -13), 1.14 (3H, d, J=7.0 Hz, H_3-5′), 0.87 (3H, t, J=7.5 Hz, H_3-4′). ^{13}C-NMR ($CDCl_3$): δ 175.9 (C-1′), 161.3 (C-2), 156.4 (C-7), 153.4 (C-8a), 143.9 (C-4), 126.8 (C-5), 114.4 (C-6), 112.7 (C-3), 112.2 (C-4a), 107.3 (C-8), 76.8 (C-11), 69.3 (C-10), 41.1 (C-2′), 26.7 (C-3′), 25.5 (C-12 or 13), 23.2 (C-9), 22.5 (C-12 or 13), 16.7 (C-5′), 11.6 (C-4′). HR-FAB-MS m/z: 353.1467. $C_{19}H_{22}O_5Na$ required 353.1365.

10-Chloro-1-heptadecene-4,6-diyne-3,8,9-triol (**3**): Amorphous powder, $[\alpha]_D^{25}$ +47.5° (c=0.1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3413, 2927, 2856, 2362, 1643, 1531, 1458, 1261, 1093, 1032, 800. UV λ_{max} nm (MeOH) (log ε): 257 (3.18), 244 (3.17). ^{1}H-NMR ($CDCl_3$): δ: 5.95 (1H, ddd, J=17.0, 10.2, 5.2 Hz, H-2), 5.49 (1H, d, J=17.0 Hz, H-1a), 5.28 (1H, d, J=10.2 Hz, H-1b), 4.96 (1H, d, J=5.2 Hz, H-3), 4.55 (1H, d, J=6.3 Hz, H-8), 4.28 (1H, m, H-10), 3.74 (1H, dd, J=6.3, 4.2 Hz, H-9), 1.83 (2H, m, H_2-11), 0.90 (3H, t, J=7.0 Hz, H_3-17). ^{13}C-NMR ($CDCl_3$): δ 135.6 (C-2), 117.6 (C-1), 78.4 (C-4), 77.7 (C-7), 75.6 (C-9), 70.7 (C-5), 70.0 (C-6), 64.5 (C-8), 64.4 (C-10), 63.5 (C-3), 34.6 (C-11), 31.8, 29.2, 29.0, 26.4, 22.7 (C-12-C-16), 14.2 (C-17). HR-FAB-MS m/z (rel. int.): 335.1375 $[M+Na]^+$. $C_{17}H_{25}ClO_3Na$ required 335.1390.

Acetylation of 3 Compound **3** (1 mg) was treated with Ac_2O (0.3 ml) and C_5D_5N (0.5 ml) at room temperature overnight. The reaction mixture was worked up in the usual way to give triacetate **3a** (0.7 mg). Compound **3a**: ^{1}H-NMR ($CDCl_3$): δ: 5.86 (1H, d, J=5.0 Hz, H-3), 5.82 (1H, m, H-2), 5.64 (1H, d, J=9.9 Hz, H-1a), 5.55 (1H, d, J=6.2 Hz, H-8), 5.34 (1H, d, J=17.1 Hz, H-1b), 5.29 (1H, m, H-9), 4.05 (1H, m, H-10), 2.17 (3H, s, –OAc), 2.10 (3H, s, –OAc), 2.08 (3H, s, –OAc), 0.87 (3H, t, J=7.1 Hz, H_3-17).

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