Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 3579–3582
Tetrahedron Letters

Diastereo- and enantioselective synthesis of a conagenin skeletal amide moiety

J. Augusto R. Rodrigues,* Paulo J. S. Moran, Cíntia D. F. Milagre and Cleber V. Ursini

Universidade Estadual de Campinas, Instituto de Química, CP 6154, 13084-971 Campinas SP, Brazil

Received 12 January 2004; revised 27 January 2004; accepted 10 March 2004

Abstract—A synthetic study was carried out to obtain epimers of a protected 2,4-dihydroxy-3-methylpentanoic ester **9**, which is a central building block of the immunomodulator (+)-conagenin **1**. The configuration of the three contiguous stereogenic centers was determined by NMR measurements and comparison with the stereogenic centers of lactone **10**.

1. Introduction

(+)-Conagenin **1** was isolated from fermentation broths of *Streptomyces roseosporus* by Ishizuka and co-workers after screening for substances that exhibit specific action on T cells by producing lymphokines as a low-molecular mass immunomodulator.[1] It was reported that **1** improved the antitumor efficacy of adriamycin and mitomycin C against murine leukemias, which suggest its potential utility for cancer chemotherapy.[2] The biological activity and the unique highly functionalized structure of (+)-conagenin has stimulated several efforts directed toward the synthesis of **1**. A first study was attempted by Sztaricskai and co-workers who obtained conagenin diastereoisomers using as starting material a functionalized D-xylose.[3] The first total synthesis of (+)-conagenin was achieved by Irie et al. starting with the asymmetric aldol reaction of propiophenone with acetaldehyde followed by in situ *syn*-selective $NaBH_4$ reduction.[4] A formal synthesis was achieved by Enders et al. in a 12-step sequence via asymmetric [2,3]-Wittig rearrangement of crotyloxyacetaldehyde-SAEP-hydrazone and the diastereoselective reduction of a methylketone.[5] A second total synthesis by Sano et al., was accomplished based on enantioselective enzymatic reaction followed by chemoselective reduction.[6] The combination of its biological activity and its unique structure of encouraged us to develop a new approach to the synthesis of amide moiety of conagenin **1** and its epimers (Fig. 1).

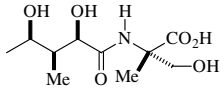


Figure 1. (+)-conagenin **1**.

2. Results and discussion

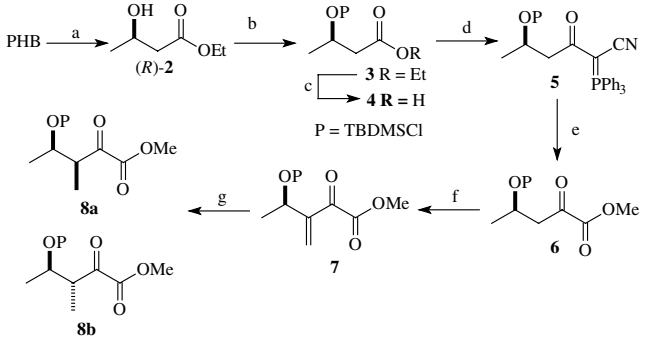
Our strategy is based on the easy and inexpensive access to poly-[(*R*)-3-hydroxybutyric] acid (PHB)[7] from the sugar cane industry, which is depolymerized to give ethyl (*R*)-(−)-3-hydroxybutanoate **2** after esterification of the corresponding acid in high yield and pure enantiomeric form (Scheme 1).[8] Protection of the hydroxyl with TBDMSCl in the presence of imidazole/DMF gave **3** in high yield. Saponification with $LiOH/H_2O$ in methanol and further acidification with HCl gave the corresponding acid **4** in 60% yield after two steps from **2**. Many methods are known for the conversion of carboxylic acids to 2-oxo esters.[9] However, we required a strategy which would maintain the stereochemical integrity of the molecule and we favored a method involving ozonolysis of β-ketocyanophosphoranes, originally described by Wasserman and Ho.[10] Treatment of the carboxylic acid **4** with (cyanomethylene)triphenylphosphorane in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and 4-dimethylaminopyridine (DMAP) followed by ozonolysis of **5** in

Keywords: Conagenin; PHB-biopolymer; β-methylene-α-keto ester; Hydrogenation.
* Corresponding author. Tel.: +55-1937883141; fax: +55-1937883023; e-mail: jaugusto@iqm.unicamp.br

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.03.062

methanol and dichloromethane[11] gave the required 2-oxo ester (*R*)-(−)-**6** in 66% yield.[12]

It was then necessary to prepare the α-keto-β-methylene ester **7** that was obtained via direct Mannich methylenation, using a methodology recently optimized in our laboratory,[13] by heating (70 °C) a mixture containing the keto ester **6** in anhydrous acetic acid with a catalytic amount of morpholine and an excess of paraformaldehyde for 3 h. Compound **7** was obtained in 35% yield as a pale yellow oil.[14] Efforts to improve the yield of **7** were unproductive. Hydrogenation of the α-keto-β-methylene ester **7** over 5% Pd/C were performed in ethyl acetate under atmospheric pressure to give *syn* and *anti* keto ester **8a/8b** in 70% yield in a ratio 93:7, respectively. Structural assignments of the isomers were determined by ^{1}H and ^{13}C NMR spectroscopy. The magnitudes of the 3-H and 4-H vicinal coupling constants of *syn* (5.1 Hz) and *anti* (7.5 Hz) isomers are consistent with those observed for β-hydroxy (or β-silyloxy)-α-methyl esters,[15] thioesters,[15a] aldehydes,[16] acids,[17] amides,[18] and ketones.[19] The ^{13}C NMR resonances of 3-C, 4-C and methyl-3-C of *syn* isomer **8a** appeared at higher fields than the respective carbons of *anti* **8b**. This behavior is in accordance with empirical observations that allow the assignment of the stereostructure of β-hydroxycarbonyl compounds possessing diastereoisomerism.[20] Similar β-silyloxycarbonyl compounds show the same pattern for ^{13}C NMR spectra.[21,15f] The stereochemical outcome of this hydrogenation can be rationalized on the basis of the Felkin[22] and the Cram[23] models. The α-ketoesters mixture **8a** and **8b** was reduced with tetrabutylammonium borohydride in methanol at −78 °C to give a diastereomeric mixture of α-hydroxyesters **9a**–**c** in 67% yield, having a ratio of 85:6:9, respectively (Scheme 2).



Scheme 1. Reagents and conditions: (a) 1,2-dichloroetane, EtOH, cat. H_2SO_4, two days (60% yield); (b) TBDMSCl, imidazole, DMF; (c) LiOH, MeOH, H_2O/HCl (60% yield, two steps); (e) Ph_3PCHCN, EDCI, DMAP, CH_2Cl_2 ; (e) O_3, MeOH, CH_2Cl_2, −78 °C (66% yield, two steps); (f) $(CH_2O)_n$, morpholine, HOAc, 70 °C (35% yield); (g) H_2, Pd–C 5%, EtOAc (70% yield, **8a**:**8b** 93:7).

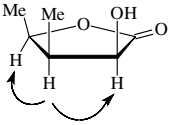


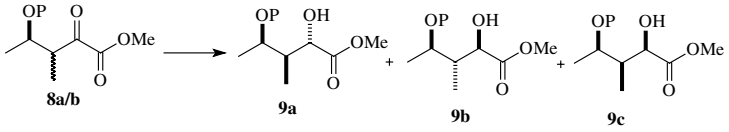
Figure 2. Selected ^{1}H–^{1}H NOE enhancements (500 MHz, ^{1}H NMR, $CDCl_3$) for **10a**.

Purification by silica gel flash chromatography eluted with hexane (95%):ethyl acetate isolated pure **9a**[24] in 53% yield and **9c**:**9b**[25] in a 9/1 ratio, as determined by NMR. Considering that **9c** has the correct *syn* stereochemistry for the three contiguous chiral centers of the amide moiety of conagenin **1**, we decided to search for other reagents to reduce **8a/8b** to improve its amount in the product ratio. Reduction with $LiBH_4$ in ethyl ether at −90 °C increased the quantity of **9c** to 41% (Scheme 2). Deprotection of **9a** with tetrabutylammonium fluoride in THF gave the lactone **10a**[26] in 70% yield. The ^{1}H NMR of **10a** shows coupling constants $J_{3,4} = 7.3$ Hz and $J_{4,5} = 4.6$ Hz that are in agreement with previous coupling constant analyses and conformational studies by MM2 calculations of substituted γ-lactones with vicinal hydrogen atoms.[27] The stereochemistry of **10a** was also revealed by NOE analysis (500 MHz, $CDCl_3$, Fig. 2). Under the same conditions, **9c** was deprotected and cyclized to lactone **10c** in high yield. The ^{1}H NMR coupling constant of **9c** for the vicinal $J_{2,3}$ (2.4 Hz) and $J_{3,4}$ (5.8 Hz) are close to those for (+)-conagenin **1**.[6] Lactone **10c**[28] shows $J_{3,4} = 10.3$ Hz and $J_{4,5} = 7.0$ Hz, also in agreement with the calculated coupling constants: $J_{3,4} = 9.8$ Hz and $J_{4,5} = 7.1$ Hz.[27] The magnitude of $J_{3,4}$ (10.3 Hz) is characteristic for hydrogens 3-H and 4-H with *trans* geometry.[16,27]

In conclusion, we have developed a new short route for the synthesis of the amide moiety of conagenin and its epimers. The β-methylene-α-keto ester **7** proved its versatility for obtaining the conagenin skeleton through hydrogenation reduction of the enone moiety with asymmetric induction. The ^{1}H NMR coupling constants and formation of the lactones allow characterization of all the isolated diastereoisomers.

Acknowledgements

We thank FAPESP for financial support and for a scholarship for C.V.U., and Dr. Gilson H. M. Dias for the use of his lab facilities.



Scheme 2. Reagents and conditions: (1) Bu_4NBH_4, MeOH, −78 °C (67% yield, **9a**:**b**:**c** 85:6:9); (2) $LiBH_4$, Et_2O, −90 °C (53% yield, **9a**:**b**:**c** 54:5:41).

References and notes

1. (a) Yamashita, T.; Iijima, M.; Nakamura, H.; Isshiki, K.; Naganawa, H.; Hattori, S.; Hamada, M.; Ishizuka, M.; Takeuchi, T. *J. Antibiot.* **1991**, *44*, 557–559; (b) Kawatsu, M.; Yamashita, T.; Osono, M.; Ishizuka, M.; Takeuchi, T. *J. Antibiot.* **1993**, *46*, 1687–1691; (c) Ishizzuka, M.; Kawatsu, M.; Yamashita, T.; Ueno, M.; Takeuchi, T. *Int. J. Immunopharmacol.* **1995**, *17*, 133–139.
2. (a) Kawatsu, M.; Yamashita, T.; Ishizuka, M.; Takeuchi, T. *J. Antibiot.* **1995**, *48*, 222–225; (b) Hamada, M.; Yamamoto, S.; Moriguchi, S.; Kishino, Y. *J. Antibiot.* **2001**, *54*, 349–353; (c) Hamada, M.; Sonotake, E.; Yamamoto, S. Morigushi, S. *J. Antibiot.* **1999**, *52*, 548–551.
3. Kovács-Kulyassa, Á.; Herczegh, P.; Sztaricskai, F. J. *Tetrahedron Lett.* **1996**, *37*, 2499–2502.
4. Hatakeyama, S.; Fukuyama, H.; Mukugi, Y.; Irie, H. *Tetrahedron Lett.* **1996**, *37*, 4047–4050.
5. Enders, D.; Bartsch, M.; Runsink, J. *Synthesis* **1999**, 243–248.
6. Sano, S.; Miwa, T.; Hayashi, K.; Nozaki, K.; Ozaki, Y.; Nagao, Y. *Tetrahedron Lett.* **2001**, *42*, 4029–4031.
7. Bachmann, B. M.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 2430–2461.
8. Seebach, D.; Beck, A. K.; Breitschuh, R.; Job K. *Org. Synth.* **1993**, *71*, 39–47.
9. For reviews of the synthesis of 2-oxo esters see: (a) Kovacs, L. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 471–496; (b) Copper, A. J. L.; Ginos, J. Z.; Meister, A. *Chem. Rev.* **1983**, *83*, 321–358.
10. Wasserman, H. H.; Ho, W.-B. *J. Org. Chem.* **1994**, *59*, 4364–4366.
11. This methodology was also employed by Bhalay, G.; Clough, S.; McLaren, L.; Sutherland, A.; Willis, C. L. *J. Chem. Soc., Perkin Trans. 1* **2000**, 901–910.
12. (*R*)-**6**: $[\alpha]_D^{20}$ − 22.4 (*c* 1.21 $CHCl_3$); IR (thin film) *v* (C=O) 1755, 1733 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 4.38 (m, 1H, 4-H), 3.85 (s, 3H, CO_2CH_3), 3.04 (dd, 1H, $J_{3,3} = 15.6$, $J_{3,4} = 7.3$, 3-*H*H), 2.87 (dd, 1H, $J_{3,3} = 15.6$, $J_{3,4} = 5.2$, 3-H*H*), 1.21 (d, 3H, $J_{4,5} = 6.1$, 5-H_3), 0.84 [s, 9H, $SiC(CH_3)_3$], 0.053 (s, 3H, $SiCH_3$), 0.017 (s, 3H, $SiCH_3$).
13. Rodrigues, J. A. R.; Siqueira-Filho, E. P.; de Mancilha, M.; Moran, P. J. S. *Synth. Commun.* **2003**, *33*, 331–340.
14. (*R*)-**7**: $[\alpha]_D^{20}$ − 27.6 (*c* 1.66, $CHCl_3$); IR (thin film) *v* (C=O) 1746, 1682 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H 6.54 (d, 1H, $J = 1.1$ Hz, =C*H*H), 6.24 (s, 1H, =CH*H*), 4.76 (q, 1H, $J_{4,5} = 6.2$ Hz, 4-H), 3.91 (s, 3H, CO_2CH_3), 1.28 (d, 3H, $J_{4,5} = 6.2$ Hz, 5-H_3), 0.91 [s, 9H, $SiC(CH_3)_3$], 0.084 (s, 3H, $SiCH_3$), 0.039 (s, 3H, $SiCH_3$). Anal. Calcd for $C_{13}H_{24}O_4Si$: C, 57.32; H, 8.88. Found: C, 57.07; H, 9.0.
15. (a) Evans, D. A.; Kozlowski, M. C.; Murry, J. A.; Burgey, C. S.; Campos, K. R.; Connell, B. T.; Staples, R. J. *J. Am. Chem. Soc.* **1989**, *121*, 669–685; (b) Slough, G. A.; Bergman, R. G.; Heathcock, C. H. *J. Am. Chem. Soc.* **1999**, *111*, 938–949; (c) Hoffmann, R. W.; Dresely, S.; Lanz, J. W. *Chem. Ber.* **1988**, *121*, 1501–1507; (d) Fráter, G.; Müller, U.; Günther, W. *Tetrahedron* **1984**, *40*, 1269–1277; (e) Scheidt, K. A.; Bannister, T. D.; Tasaka, A.; Wendt, M. D.; Savall, B. M.; Fegley, G. J.; Roush, W. R. *J. Am. Chem. Soc.* **2002**, *124*, 6981–6990; (f) Mateus, C. R.; Feltrin, M. P.; Costa, A. M.; Coelho, F.; Almeida, W. P. *Tetrahedron* **2001**, *57*, 6901–6908; (g) Keck, G. E.; Kachensky, D. F.; Enholm, E. J. *J. Org. Chem.* **1985**, *50*, 4317–4325.
16. Barrett, A. G. M.; Lebold, S. A. *J. Org. Chem.* **1991**, *56*, 4875–4884.
17. Renauld, P.; Seebach, D. *Helv. Chim. Acta* **1986**, *69*, 1704–1710.
18. Evans, D. A.; Ratz, A. M.; Huff, B. E.; Sheppard, G. S. *J. Am. Chem. Soc.* **1995**, *117*, 3448–3467.
19. Enders, D.; Bartsch, M.; Runsink, J. *Synthesis* **1999**, 243–248.
20. Heathcock, C. H.; Pirrung, M. C.; Sohn, J. E. *J. Org. Chem.* **1979**, *44*, 4294–4299.
21. Hoffmann, R. W.; Weidmann, U. *Chem. Ber.* **1985**, *118*, 3966–3979.
22. Jackson, R. F. W.; Standen, S. P.; Clegg, W.; McCamley, A. *Tetrahedron Lett.* **1992**, *33*, 6197–6200.
23. Augustine, R. L. *Catal. Today* **1997**, *37*, 419–440.
24. Methyl (2*S*,3*S*,4*R*)-4-(*t*-butyldimethylsilyloxy)-2-hydroxy-3-methylpentanoate **9a**: IR (thin film) 3476 (br) and 1738 cm^{-1}; $[\alpha]_D^{23}$ − 4.0 (*c* 1.03, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 4.16 (dd, 1H, $J_{2,3} = 7.0$ Hz, $J_{2,2\text{-OH})} = 6.4$ Hz, 2-H), 4.09 (dq, 1H, $J_{4,5} = 6.4$ Hz, $J_{3,4} = 3.0$ Hz, 4-H), 3.88 (d, 1H, $J_{2,2\text{-OH}} = 6.1$ Hz, O*H*), 3.77 (s, 3H, CO_2CH_3), 1.92 (dquint, 1H, $J_{3,3\text{-methyl}} = 7.0$ Hz, $J_{2,3} = 7.0$ Hz, $J_{3,4} = 3.0$ Hz, 3-H), 1.18 (d, 3H, $J_{4,5} = 6.4$ Hz, 5-H_3), 0.93 (d, 3H, $J_{3,3\text{-methyl}} = 7.0$ Hz 3-C–CH_3), 0.90 [s, 9H, $SiC(CH_3)_3$], 0.11 (s, 3H, $SiCH_3$), 0.09 (s, 3H, $SiCH_3$); ^{13}C (^1H) NMR, (125.7 MHz, $CDCl_3$) δ_C 174.59 (1-C), 74.73 (2-C or 4-C), 70.42 (4-C or 2-C), 51.92 (O–CH_3), 42.74 (3-C), 25.78 [$SiC(CH_3)_3$], 19.99 (SiC), 17.93 (SiC), 11.22 (3-CCH_3), −4.24 (SiCH_3), −5.03 (SiCH_3). Anal. Calcd for $C_{13}H_{28}O_4Si$ (276.44): C, 56.48; H, 10.21. Found: C, 56.44; H, 10.47.
25. **9c**/**9b**: 9:1 ratio, IR (thin film): *v* (OH) 3500 (br s), *v* (C=O) 1738 cm^{-1} (s) $[\alpha]_D^{23}$ − 70 (*c* 0.22, $CHCl_3$)Methyl (2*R*,3*S*,4*R*)-4-(*tert*-butyldimethylsilyloxy)-2-hydroxy-3-methylpentanoate **9c**: ^1H NMR (500 MHz, $CDCl_3$) δ_H 4.45 (dd, 1H, $J_{2,3} = 2.4$ Hz, $J_{2,2\text{-OH}} = 4.3$ Hz, 2-H) 3.93 (apparent quint, 1H, $J_{4,5} = 6.1$ Hz, $J_{3,4} = 5.8$ Hz, 4-H), 3.79 (s, 3H, CO_2CH_3), 3.05 (d, 1H, $J_{2,2\text{-OH}} = 4.3$ Hz, O*H*), 1.95 (ddq, 1H, $J_{3,3\text{-methyl}} = 7.0$ Hz, $J_{2,3} = 2.4$ Hz, $J_{3,4} = 5.8$ Hz, 3-H), 1.22 (d, 3H, $J_{4,5} = 6.1$ Hz, 5-H_3), 0.90 [s, 9H, $SiC(CH_3)_3$], 0.88 (d, 3H, $J_{3,3\text{-methyl}} = 7.0$ Hz, 3-C–CH_3), 0.087 (s, 3H, $SiCH_3$), 0.081 (s, 3H, $SiCH_3$); $^{13}C\{^1H\}$ NMR. (125.7 MHz, $CDCl_3$) δ_C 175.27 (1-C), 72.29 (2-C or 4-C), 71.26 (4-C or 2-C), 52.45 (O-CH_3), 43.44 (3-C), 25.81 [$SiC(CH_3)_3$], 21.06 (5-C), 17.98 (Si*C*), 8.94 (3-CCH_3), −4.11 (Si–CH_3), −4.90 (Si–CH_3).
Methyl (2*R*,3*R*,4*R*)-4-(*tert*-butyldimethylsilyloxy)-2-hydroxy-3-methylpentanoate **9b**: ^1H NMR δ_H (500 MHz, $CDCl_3$): 4.21 (dd, 1H, $J_{2,3} = 4.3$ Hz, $J_{2,2\text{-OH}} = 5.5$ Hz, 2-H) 3.89 (apparent quint, 1H, $J = 6.4$ Hz, 4-H), 3.78 (s, 3H, CO_2CH_3), 3.37 (d, 1H, $J_{2,2\text{-OH}} = 5.9$ Hz, O*H*), 2.00 (m, 1H, $J = 7.0$ Hz, $J = 4.6$ Hz, 3-H), 1.16 (d, 3H, $J_{4,5} = 6.1$ Hz, 5-H_3), 0.97 (d, 3H, $J_{3,3\text{-methyl}} = 7.0$ Hz, 3-C–CH_3), 0.89 [s, 9H, $SiC(CH_3)_3$], 0.077 (s, 3H, $SiCH_3$), 0.071 (s, 3H, $SiCH_3$); $^{13}C\{^1H\}$ NMR. δ_C (125.7 MHz, $CDCl_3$): 175.04 (1-C), 73.57 (2-C or 4-C), 70.84 (4-C or 2-C), 52.08 (O-CH_3), 44.31 (3-C), 25.86 [$SiC(CH_3)_3$], 21.55 (5-C), 17.98 (Si*C*), 13.52 (3-CCH_3), −4.34 (Si–CH_3), −4.73 (Si–CH_3).
26. (3*S*,4*S*,5*R*)-3-hydroxy-4,5-dimethyl-tetrahydrofuran-2-one **10a**: $[\alpha]_D^{23}$ +17 (*c* 0.080, $CHCl_3$); IR (nujol): *v* (OH) 3440 cm^{-1} (br, s), *v* (C=O) 1766 cm^{-1} (s); ^1H NMR δ_H (500 MHz, $CDCl_3$): 4.58 (dq, 1H, $J_{4,5} = 4.6$ Hz, $J_{5,5\text{-methyl}} = 6.4$ Hz, 5-H), 4.57 (d, 1H, $J_{3,4} = 7.3$ Hz, 3-H), 2.70 (apparent dquint and br s, 2H, $J = 7.3$ Hz, $J_{4,5} = 4.6$ Hz, 4-H and –O*H*), 1.38 (d, 3H, $J_{5,5\text{-methyl}} = 6.4$ Hz, 5-CH_3), 0.95 (d, 3H, $J_{4,4\text{-methyl}} = 7.3$ Hz, 4-CH_3); $^{13}C\{^1H\}$ NMR. δ_C (125.7 MHz, $CDCl_3$): 177.32 (2-C), 76.20 (3-C or 5-C), 71.80 (5-C or 3-C), 39.24 (4-C), 15.45 (5-CCH_3),6.37 (4-CCH_3).
27. Jaime, C.; Segura, C.; Dinarés, I.; Font, J. *J. Org. Chem.* **1993**, *58*, 154–158.
28. (3*R*,4*S*,5*R*)-3-hydroxy-4,5-dimethyl-tetrahydrofuran-2-one **10c**: mp 69–72 °C; $[\alpha]_D^{23}$ + 60 (*c* 0.027, $CHCl_3$); IR

(nujol): ν (OH) 3400 cm^{-1} (br, s), ν (C=O) 1760 cm^{-1} (s); ^1H NMR δ_H (300 MHz, $CDCl_3$): 4.72 (quint, 1H, $J_{4,5} = 7.0$ Hz, $J_{5,5\text{-methyl}} = 7.0$ Hz, 5-H), 4.11 (d, 1H, $J_{3,4} = 10.3$ Hz, 3-H), 2.58 (m, 2H, 4-H and –O*H*), 1.31 (d, 3H, $J_{5,5\text{-methyl}} = 6.6$ Hz, 5-CH_3), 1.21 (d, 3H, $J_{4,4\text{-methyl}} = 6.9$ Hz, 4-CH_3).