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Synthesis and Biological Evaluation of (±)-Dinemasone C and Analogues

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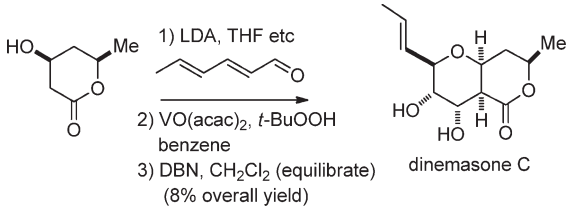


Dinemasone C was prepared in three steps (8% overall yield) from *cis*-tetrahydro-4-hydroxy-6-methyl-2-pyrone by aldol reaction with 2,4-hexadienal, epoxidation followed by cyclization, and epimerization of the ring fusion. Dinemasone C, *epi*-dinemasone C, anhydrodinemasone BC, and *nor*-dinemasone B are active against bacteria, including *Legionella pneumophila* Corby, algae, and fungi.

Krohn and co-workers recently isolated dinemasones B (**1**) and C (**2**) from a culture of the endophytic fungus *Dinemasporium strigosum*, which was isolated from the roots of *Calystegia sepium* (see Figure 1).[1] The major isomer, dinemasone B (**1**), is active against the Gram-positive bacterium *Bacillus megaterium*, the fungus *Microbotryum violaceum*, and the alga *Chlorella fusca*. The minor isomer, dinemasone C (**2**), was characterized as a mixture of dinemasone B and C diacetates and was therefore not tested for biological activity. The structure of **1** is similar to that of the moderately biologically active fusidilactone B (**3**), which differs in the size of the lactone ring, the side chain, and the absolute stereochemistry, and was previously isolated by Krohn and co-workers from an endophytic fungus *Fusidium sp.*[2] Other members of this family include TAN-2483A (**4**) with strong c-src kinase inhibitory action, which was isolated by a Takeda Chemical Industries group from the filamentous fungus NF 2329,[3] and waol-A (**5**) with broad spectrum antitumor activity, which was isolated by Mizoue and co-workers from another fungus, *Myceliophthora lutea* TF-0409.[4]

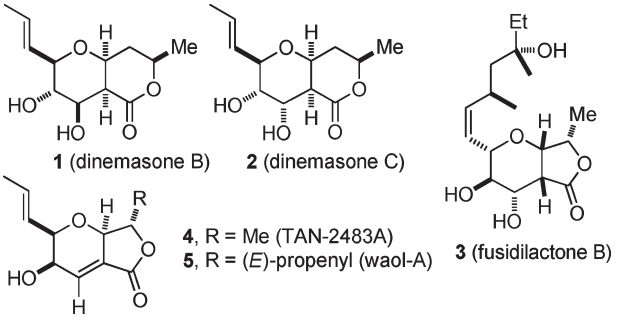
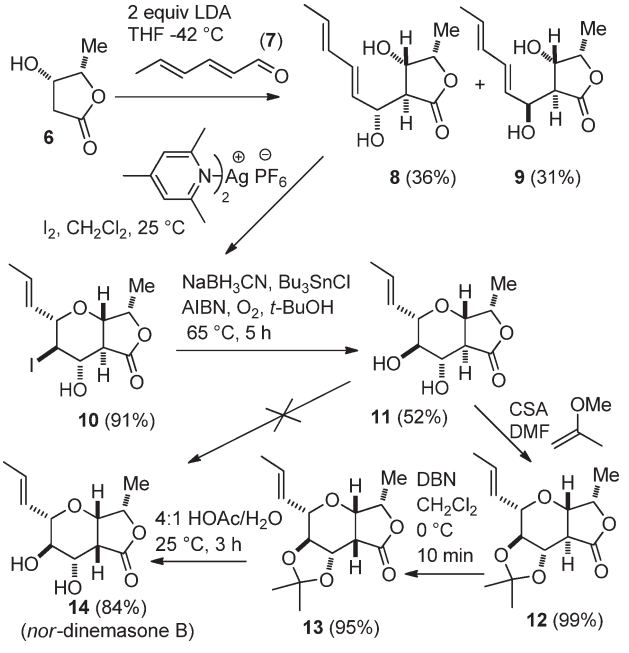


FIGURE 1. Structures of dinemasones B (**1**) and C (**2**), fusidilactone B (**3**), TAN-2483A (**4**), and waol-A (**5**).

We recently reported short, efficient syntheses of TAN-2483A (**4**), waol-A (**5**), and the ring system of fusidilactone B (**3**).[5] In the latter synthesis, we converted hydroxy lactone **6** to the dianion with LDA at −42 °C and treated it with 2,4-hexadienal (**7**) to give aldol adducts **8** (36%) and **9** (31%) (see Scheme 1). The major adduct **8** was subjected to iodoetherification with iodine and bis(*sym*-collidine)$AgPF_6$ to give iodo alcohol **10** in 91% yield. Radical deiodination of **10** in the presence of oxygen afforded the desired diequatorial alcohol **11** in 52% yield. The diol was then protected to give acetonide **12** in 99% yield. Epimerization of the acidic proton adjacent to the carbonyl group with DBN in CH_2Cl_2 afforded the cis-fused lactone **13** in 95% yield. In our previous study, we cleaved the double bond to give an aldehyde and then used a Wittig reaction to prepare a cis double bond similar to that in fusidilactone B (**3**) prior to acidic cleavage of the acetonide.

SCHEME 1. Synthesis of *nor*-Dinemasone B (14)



(1) Krohn, K.; Sohrab, Md. H.; van Ree, T.; Draeger, S.; Schulz, B.; Antus, S.; Kurtán, T. *Eur. J. Org. Chem.* **2008**, 5638–5646.

(2) (a) Krohn, K.; Biele, C.; Drogies, K.-H.; Steingröver, K.; Aust, H.-J.; Draeger, S.; Schulz, B. *Eur. J. Org. Chem.* **2002**, 2331–2336. (b) Qin, S.; Krohn, K.; Flörke, U.; Schulz, B.; Draeger, S.; Pescitelli, G.; Salvadori, P.; Antus, S.; Kurtán, T. *Eur. J. Org. Chem.* **2009**, 3279–3284.

(3) Hayashi, K.; Takizawa, M.; Noguchi, K. Japanese Patent 10,287,679, 1998; *Chem. Abstr.* **1999**, *130*, 3122e.

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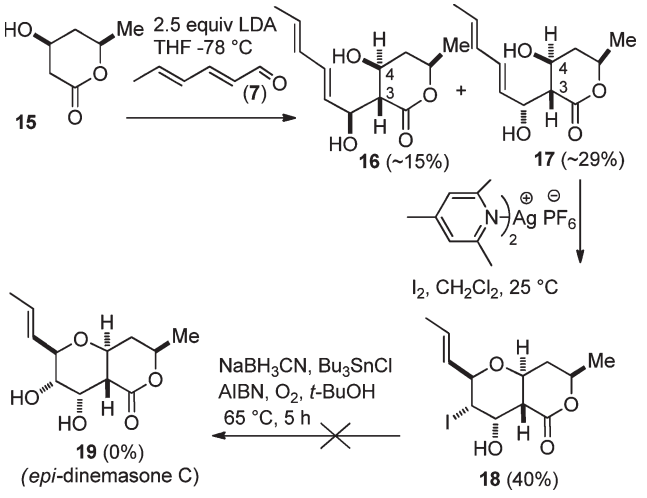
Acetonide **13** is a protected version of *nor*-dinemasone B (**14**), which differs from dinemasone B (**1**) only by the absence of a methylene group in the lactone ring. Since the route to **13** is very short, we decided to prepare **14** for evaluation of its biological activity. Attempted epimerization of **11** without protection of the diol using DBN in CH_2Cl_2 at 0 °C gave a complex mixture containing little **14**.[6] Therefore, acetonide **13** was hydrolyzed with 4:1 HOAc/H_2O to give *nor*-dinemasone B (**14**) in 84% yield.

We tried to apply this chemistry to the synthesis of dinemasones B (**1**) and C (**2**) starting with δ-lactone **15**[7] rather than γ-lactone **6** (see Scheme 2). Conversion of **15** to the dianion with LDA at −78 °C and treatment with 2,4-hexadienal (**7**) afforded a difficultly separable 1:2 mixture of aldol adducts **16** and **17** in 44% yield. The 9.2 Hz coupling constant between H-3 and H-4 established that the hexadienyl and hydroxy side chains are trans in both isomers. The stereochemistry of the side-chain hydroxy group was established by conversion of the major diastereomer **17** initially to bicyclic lactone **18** and eventually to **19** and **2**.

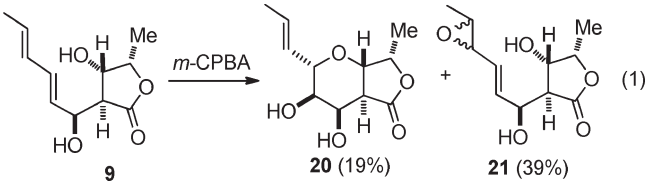
SCHEME 2. Synthesis of Iodo Alcohol 18



Unfortunately, this aldol reaction was not as successful as that with γ-lactone **6**, which proceeded in 67% yield at −42 °C. This aldol reaction gave a complex mixture at −42 °C and proceeded in only 44% yield at −78 °C. Adduct **16** needed for the synthesis of dinemasone B (**1**) was the minor adduct, whereas isomer **8** needed for the synthesis of *nor*-dinemasone B (**14**) was the major adduct. Finally, aldol adducts **8** and **9** were easily separable, whereas **16** and **17** could not be separated on a preparative scale.

Iodoetherification of the mixture of **16** and **17** with bis-(*sym*-collidine)$AgPF_6$ (1.5 equiv) and I_2 (1.2 equiv) in CH_2Cl_2 for 3 h at 25 °C afforded iodo alcohol **18** in 40% yield as the only isolable product. In our previous studies,[5] we noted that iodoetherification proceeds much more effectively with the aldol adduct that leads to the iodoether with an axial alcohol. Therefore, it is not surprising that iodoetherification proceeds only with the major aldol adduct **17** to give **18**. Unfortunately, we were unable to convert **18** to *epi*-dinemasone C (**19**) using the conditions that were successful for the conversion of **10** to **11**. Use of TEMPO instead of oxygen to trap the radical was also unsuccessful.[8]

We previously found that epoxidation of **9** with *m*-CPBA in CH_2Cl_2 at 0 °C gave cis bicyclic diol **20** in 19% yield and a mixture of epoxides **21** on the distal double bond in 39% yield (see eq 1).[5b] A similar sequence would convert the major aldol adduct **17** to *epi*-dinemasone C (**19**) (see Scheme 3). For this purpose, we needed to convert allylic alcohol **17** to erythro epoxy alcohol **24**, which can cyclize through a chairlike transition state to give *epi*-dinemasone C (**19**). Cyclization should occur selectively at the more reactive allylic position to give a tetrahydropyran, rather than at the other epoxide carbon to give a tetrahydrofuran. Formation of the tetrahydrofuran would be a major side reaction in the absence of the double bond, which activates the allylic position of the epoxide toward an S_N2 reaction. Under erythro selective epoxidation conditions, allylic alcohol **16** will give erythro epoxy alcohol **22**, which would have to cyclize through a high energy boatlike transition state to give **23**. We also needed to choose oxidation conditions that would selectively oxidize the allylic alcohol double bond, rather than the distal double bond as was observed in the formation of **21** from **9** using *m*-CPBA.



Adam has studied the regioselectivity of the epoxidation of 1-methylgeraniol, in which the two double bonds differ only in their proximity to the alcohol.[9] The allylic alcohol double bond is epoxidized with high (> 95%) selectivity using *tert*-butyl hydroperoxide (TBHP) and either $VO(acac)_2$ or Ti(O-*i*-Pr)$_4$. Use of *m*-CPBA gave a 1:1 mixture of regioisomeric epoxides analogously to the epoxidation of **9** with *m*-CPBA. Other oxidants epoxidized the isolated double bond selectively. Adam also studied the stereoselectivity of the epoxidation of 2,2-dimethyl-4-hexen-3-ol, an allylic alcohol analogous to **16** and **17**.[10] He obtained an 82:18 mixture favoring the erythro epoxide with TBHP and $VO(acac)_2$ in benzene, whereas the erythro epoxide was the minor isomer with Ti(O-*i*-Pr)$_4$ (43:57), *m*-CPBA (47:53), and dimethyldioxirane (48:52). These results suggested that use of $VO(acac)_2$ and

(4) (a) Nozawa, O.; Okazaki, T.; Sakai, N.; Komurasaki, T.; Hanada, K.; Morimoto, S.; Chen, Z.-X.; He, B.-M.; Mizoue, K. *J. Antibiot*. **1995**, *48*, 113–118. (b) Nazawa, O.; Okazaki, T.; Morimoto, S.; Chen, Z.-X.; He, B.-M.; Mizoue, K. *J. Antibiot*. **2000**, *53*, 1296–1300.

(5) (a) Gao, X.; Snider, B. B. *Org. Lett*. **2003**, *5*, 451–454. (b) Gao, X.; Snider, B. B. *J. Org. Chem*. **2004**, *69*, 5517–5527.

(6) The enolate formed from acetonide **12** cannot undergo elimination because the trans-fused acetonide locks the oxygen in a conformation that is orthogonal to the π electrons of the enolate. This constraint is absent with the alcohol groups of **11**.

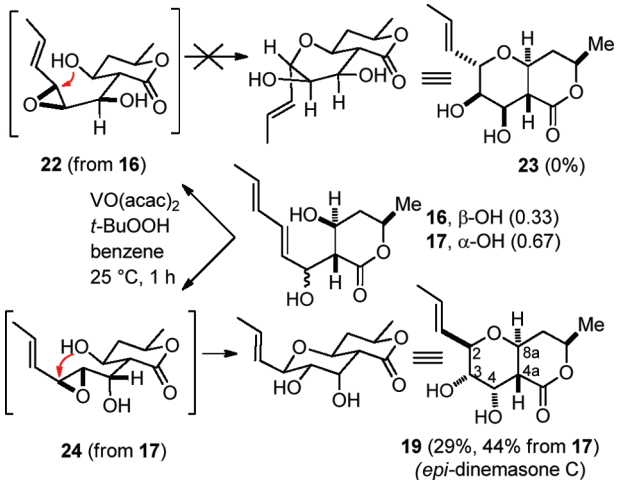
(7) Prepared in a single step by hydrogenation of 4-hydroxy-6-methyl-2-pyrone over Raney nickel. (a) Bacardit, R.; Moreno-Mañas, M. *Tetrahedron Lett*. **1980**, *21*, 551–554. (b) Fehr, M. J.; Consiglio, G.; Scalone, M.; Schmid, R. *J. Org. Chem*. **1999**, *64*, 5768–5776.

(8) Vogler, T.; Studer, A. *Synthesis* **2008**, 1979–1993.

(9) Adam, W.; Michell, C. M.; Paredes, R.; Smerz, A. K.; Veloza, L. A. *Liebigs Ann. Chem*. **1997**, 1365–1369.

(10) Adam, W.; Corma, A.; Reddy, T. I.; Renz, M. *J. Org. Chem*. **1997**, *62*, 3631–3637. See also: Mihelich, E. D. *Tetrahedron Lett*. **1979**, 4729–4732.

SCHEME 3. Synthesis of *epi*-Dinemasone C (19)



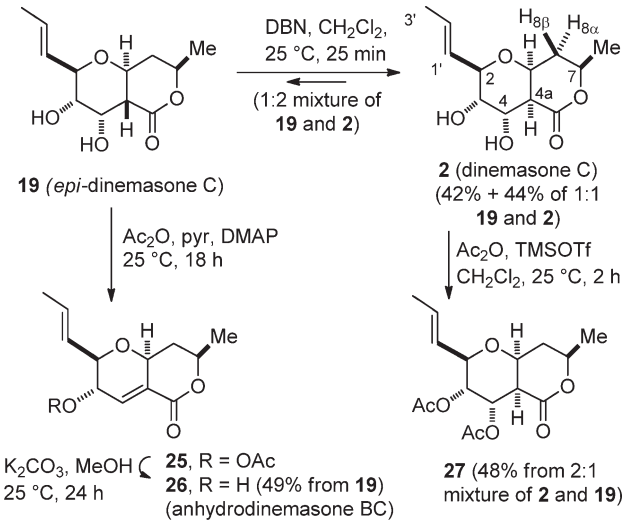
TBHP would give us both the optimal stereo- and regioselectivity.[11]

We were delighted to find that epoxidation of the 1:2 mixture of **16** and **17** with $VO(acac)_2$ and *t*-BuOOH in benzene[12] produced *epi*-dinemasone C (**19**) as the only isolable product in 29% (44% from **17**) yield. The large coupling constants between hydrogens H-2 and H-3 (8.0 Hz) and between H-4a and H-8a (10.6 Hz) established that these four hydrogens are all axial. The small coupling constants between H-3 and H-4 and between H-4 and H-4a established that H-4 is equatorial. Presumably, epoxidation forms erythro epoxy alcohol **24**, which cyclizes under the reaction conditions. The modest yield probably results from incomplete stereo- and regioselectivity in the epoxidation and from epoxide cleavage with external nucleophiles. Erythro epoxy alcohol **22** is probably formed from **16** but should react with external nucleophiles, rather than cyclizing through a high energy boatlike transition state. Neither **19** nor stereoisomeric bicyclic products were obtained by epoxidation of the mixture of **16** and **17** with *m*-CPBA in CH_2Cl_2 at 0 or 25 °C, TBHP and $Ti(O\text{-}i\text{-}Pr)_4$ in CH_2Cl_2 at 25 °C, or TBHP and $Mo(CO)_6$ in CH_2Cl_2 at 25 °C.

We were pleased to find that epimerization of the ring fusion of **19** using 2.5 equiv of DBN in CH_2Cl_2 for 25 min at 25 °C gave a 2:1 equilibrium mixture of dinemasone C (**2**) and *epi*-dinemasone C (**19**) containing only 7% of anhydrodinemasone BC (**26**) (see Scheme 4). The 3.6 Hz coupling constant between H-4a and H-8a of **2** indicates that the ring fusion is cis. The coupling constants of 9.2 Hz between H-8a and H-8α and the 12.0 Hz coupling constant between H-7 and H-8*β* established that the lactone of dinemasone C (**2**) adopts a boat conformation as reported by Krohn for dinemasone B (**1**).[1]

Krohn characterized dinemasone C (**2**) by conversion of a mixture of dinemasones B (**1**) and C (**2**) to the diacetates with Ac_2O, pyridine, and DMAP in low yield.[1] Under these conditions, we found that the major product from **2** was anhydrodinemasone BC acetate (**25**), which was presumably formed

SCHEME 4. Synthesis of Dinemasone C (2) and Anhydrodinemasone BC (26)



by elimination of acetate from the enolate of the desired diacetate **27**. Acidic acetylation conditions[13] utilizing Ac_2O (3 equiv) and TMSOTf (0.2 equiv) converted the 2:1 mixture of **2** and **19** to a readily separable mixture of diacetate **27** (48%), with ^1H and ^{13}C NMR spectral data identical to those reported by Krohn,[1] and the trans-fused epimer (21%).

The biological activity of TAN-2483A (**4**) and waol-A (**5**) may result from their ability to act as Michael acceptors. Dehydration of dinemasones B (**1**) and C (**2**) might be occurring under biological conditions to give anhydrodinemasone BC (**26**), which could be responsible for the biological activity. We therefore converted **19** to **26** for biological evaluation. Treatment of **19** with Ac_2O, pyridine, and DMAP in CH_2Cl_2 at 25 °C for 18 h provided anhydrodinemasone BC acetate (**25**), which was hydrolyzed with K_2CO_3 in MeOH at 25 °C for 24 h to provide anhydrodinemasone BC (**26**) in 49% yield from **19**.

The activities of *nor*-dinemasone B (**14**), aldol adduct **17**, *epi*-dinemasone C (**19**), dinemasone C (**2**), and anhydrodinemasone BC (**26**) were tested in an agar diffusion assay[14] for antibacterial (*Bacillus megaterium* and *Escherichia coli*), antifungal (*Microbotryum violaceum*), and antialgal activity (*Chlorella fusca*) (see Table 1). All five compounds were active against all four test organisms. Dinemasone C (**2**) is slightly more active than epimer **19**. The conjugated double bond of **26** does not lead to improved biological activity and even the simple aldol adduct **17** is active. Several of the compounds were more active against Gram-negative *E. coli* than against Gram-positive *B. megaterium*, which is somewhat surprising because it is usually more difficult to inhibit Gram-negative than Gram-positive bacteria. We therefore evaluated the activity of these compounds against the clinically important Gram-negative bacterium *Legionella pneumophila* Corby (see Table 2). Dinemasone C and *epi*-dinemasone C were about 30−40% as active as kanamycin in an agar diffusion assay, suggesting that these are good lead structures for further development.

(11) For the selective formation of an erythro epoxy alcohol from a 2,4-dienol, see: Conrow, R. E. *Org. Lett.* **2006**, *8*, 2441–2443.
(12) Sharpless, K. B.; Verhoeven, T. R. *Aldrichim. Acta* **1979**, *12*, 63–73.
(13) Procopiou, P. A.; Baugh, S. P. D.; Flack, S. S.; Inglas, G. G. A. *J. Org. Chem.* **1998**, *63*, 2342–2347.
(14) Schulz, B.; Sucker, J.; Aust, H.-J.; Krohn, K.; Ludewig, K.; Jones, P. G.; Döring, D. *Mycol. Res.* **1995**, *99*, 1007–1015.

TABLE 1. Biological Activity of *nor*-Dinemasone B (14), Aldol Adduct 17, *epi*-Dinemasone C (19), Dinemasone C (2), and Anhydrodinemasone BC (26) against Microbial Test Organisms in an Agar Diffusion Assay at a Concentration of 50 μg per Filter Disc[a]

compd	antibacterial (*Ec*)[b]	antibacterial (*Bm*)[b]	antifungal (*Mb*)[b]	antialgal (*Chl*)[b]
14	10	10	10 pi[c]	10
17	9	7 pi[c]	7	7
19	10	7 pi[c]	7	9
2	12	6 pi[c]	9	10
26	9	7 pi[c]	7	9
penicillin	14	18	0	0
tetracycline	18	18	0	10 pi[c]
nystatin	0	0	20	0
actidione	0	0	50	35
acetone	0	0	0	0

[a]Values given are the radius of the zone of inhibition in mm. [b]*Escherichia coli* (*Ec*), *Bacillus megaterium* (*Bm*), *Microbotryum violaceum* (*Mb*), and *Chlorella fusca* (*Chl*). [c]Partial inhibition, i.e., there was some growth within the zone of inhibition.

TABLE 2. Activity of *nor*-Dinemasone B (14), Aldol Adduct 17, *epi*-Dinemasone C (19), Dinemasone C (2), and Anhydrodinemasone BC (26) against *Legionella pneumophila* Corby in an Agar Diffusion Assay at Concentrations of 100 and 200 μg per Filter Disc[a]

compd	100 μg/disc	200 μg/disc
14	0	7 pi[b]
17	0	7 pi[c]
19	6 + 0.5 pi[b]	6 + 5 pi[b]
2	10 pi[b]	7 + 5 pi[b]
26	0	10 pi[b]
kanamycin	nt	20
acetone	0	0

[a]Values given are the radius of the zone of inhibition in mm. [b]Partial inhibition, i.e., there was some growth within the zone of inhibition.

In conclusion, we have developed a practical three-step route from lactone **15** to dinemasone C (**2**). Although the yields of the individual steps are modest, the overall yield is 8% because of the brevity of the sequence. These compounds show antibacterial, antifungal, and antialgal properties.

Experimental Section

(2*R*,3*S*,4*S*,4a*R*,7*R*,8a*S*)-*rel*-Hexahydro-3,4-dihydroxy-7-methyl-2-(1*E*)-1-propen-1-yl-2*H*,5*H*-pyrano[4,3-*b*]pyran-5-one (*epi*-Dinemasone C, 19). Vanadyl acetylacetonate (14.5 mg, 0.0566 mmol) was added to a solution of the 2:1 mixture of diols **17** and **16** (64.0 mg, 0.283 mmol) in benzene (9 mL). *t*-BuOOH (57 μL, 5.0−6.0 M in nonane, 0.340 mmol) was added dropwise, and the mixture was stirred at 25 °C under nitrogen for 1 h. Additional *t*-BuOOH (14 μL, 5.0−6.0 M in nonane, 0.0849 mmol) was added to the reaction. The reaction was stirred for 1 h and treated with 10% aqueous $Na_2S_2O_3$ solution (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. Flash chromatography of the residue on MeOH-deactivated silica gel (2:1 hexanes/EtOAc) yielded 20 mg (29%) of **19** as a colorless gum as the only isolable product: ^{1}H NMR 5.92 (dq, 1, J = 15.2, 6.4, H-2′), 5.52 (ddq, 1, J = 15.2, 8.2, 1.6, H-1′), 4.67 (br s, 1, H-4), 4.52−4.45 (m, 1, H-7), 4.06 (ddd, 1, J = 12.0, 10.6, 3.6, H-8a), 3.94 (dd, 1, J = 8.2, 8.0, H-2), 3.38 (br d, 1, J = 8.0, H-3), 2.52 (br s, OH), 2.35−2.29 (m, 2, H-4a, H-8eq), 2.26 (br s, 1, OH), 1.78 (dd, 3, J = 6.4, 1.6, H-3′), 1.71 (ddd, 1, J = 12.0, 12.0, 12.0, H-8ax), 1.44 (d, 3, J = 6.4, H-7-Me); ^{13}C NMR 170.1, 132.1, 128.1, 77.2, 75.1, 71.4, 67.2, 65.5, 50.5, 37.1, 22.0, 18.1; IR (neat) 3436, 2979, 2934, 1732, 1402, 1212, 1184, 1109, 1092; MS (70 eV) m/z = 224 (M^+, 4), 227 (4), 213 (3), 154 (8), 113 (55), 95 (20), 85 (63), 84 (100), 71 (25); HRMS (EI^+) calcd for $C_{12}H_{18}O_5$ (M^+) 242.1154, found 242.1152.

(2*R*,3*S*,4*S*,4a*S*,7*R*,8a*S*)-*rel*-Hexahydro-3,4-dihydroxy-7-methyl-2-(1*E*)-1-propen-1-yl-2*H*,5*H*-pyrano[4,3-*b*]pyran-5-one (Dinemasone C, 2). DBN (54 μL, 0.44 mmol) was added dropwise to a solution of *epi*-dinemasone C (**19**) (42.4 mg, 0.175 mmol) in CH_2Cl_2 (1 mL). The reaction was stirred at 25 °C for 25 min, diluted with CH_2Cl_2 (10 mL), and washed with 2 M HCl (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL), and the combined organic layers were dried over $MgSO_4$, and concentrated. Flash chromatography of the residue on MeOH-deactivated silica gel (4:1 hexanes/EtOAc) yielded 3.2 mg (7%) of **26** followed by 19.8 mg (44%) of a 1:1 mixture of **19** and **2**, followed by 17.7 mg (42%) of pure **2** as a white solid: ^{1}H NMR 5.88 (dq, 1, J = 15.6, 6.4, H-2′), 5.42 (dd, 1, J = 15.6, 8.4, H-1′), 4.61 (br s, 1, H-4), 4.48 (ddd, 1, J = 9.2, 3.6, 3.4, H-8a), 4.33−4.28 (m, 1, H-7), 3.93 (dd, 1, J = 9.2, 8.4, H-2), 3.69 (ddd, 1, J = 9.2, 3.6, 3.3, H-3), 2.89 (dd, 1, J = 3.6, 3.4, H-4a), 2.73 (br s, OH), 2.42 (ddd, 1, J = 15.2, 9.2, 3.3, H-8α), 1.86 (d, 1, J = 3.6, OH), 1.76 (d, 3, J = 6.4, H-3′-Me), 1.68 (ddd, 1, J = 15.2, 12.0, 3.4, H-8β), 1.39 (d, 3, J = 6.0, H-7-Me); ^{13}C NMR 171.2, 132.6, 128.2, 77.2, 72.3, 68.0, 67.0, 66.1, 45.4, 36.7, 20.5, 18.0; IR (neat) 3423, 2920, 1727, 1388, 1213, 1078, 1048; MS (70 eV) m/z = 242 (M^+, 2), 227 (3), 205 (25), 113 (100), 95 (25), 71 (40); HRMS (EI^+) calcd for $C_{12}H_{18}O_5$ (M^+) 242.1154, found 242.1148.

Acknowledgment. We are grateful to the National Institutes of Health (GM-50151) for support of this work.

Supporting Information Available: Additional experimental procedures and copies of ^{1}H and ^{13}C NMR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.