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Dhilirolides E−N, Meroterpenoids Produced in Culture by the Fungus *Penicillium purpurogenum* Collected in Sri Lanka: Structure Elucidation, Stable Isotope Feeding Studies, and Insecticidal Activity

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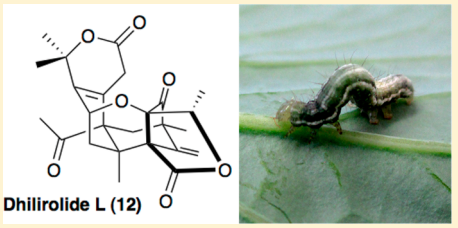
Supporting Information

ABSTRACT: Extracts of laboratory cultures of the fungus *Penicillium purpurogenum* obtained from rotting fruit of the tree *Averrhoa bilimbi* growing in Sri Lanka have yielded 10 new meroterpenoids, dhilirolides E−N (**5**−**14**). The structures of the new dhilirolides have been elucidated by analysis of spectroscopic data and a single-crystal X-ray diffraction analysis of dhilirolide L (**12**). Dhilirolides A−N (**1**−**14**) represent the four unprecedented and rearranged dhilirane, isodhilirane, 14,15-dinordhilirane, and 23,24-dinorisodhilirane meroterpenoid carbon skeletons. Stable isotope feeding studies have confirmed the meroterpenoid biogenetic origin of the dhilirolides and provided support for a proposed genesis of the new carbon skeletons. Dhilirolide L (**12**) showed significant feeding inhibition and sublethal developmental disruption in the cabbage looper *Trichoplusia ni*, an important agricultural pest, at low concentrations.



INTRODUCTION

Plants harbor many fungi on their surfaces and inside their cells.[1−4] Interactions between the host plants and fungi run the gamut from the inconsequential effects of transient saprophytes to stable mutualistic relationships, where both partners benefit from the association, and pathogenic relationships, where the fungus overwhelms the plant's defenses leading to a disease state that can result in localized plant tissue damage or even death.[5,6] Fungi are often uniquely adapted to the niche habitats of their specific plant hosts.[7−9] Sri Lanka has a high rate of endemic speciation in both plants and microbes,[10] which offers great potential for the discovery of plant-associated fungi that can produce novel secondary metabolites with medicinal or agricultural utility.[11−13] As part of an ongoing program aimed at exploring the secondary metabolites of fungi obtained from Sri Lankan plants, we have investigated the fungus *Penicillium purpurogenum* isolated from fruit of the tree *Averrhoa bilimbi*.[14] The mature fruit of *A. bilimbi* is susceptible to infection by *P. purpurogenum*, which can lead to complete decay of individual pieces of fruit.

We recently reported that cultures of *P. purpurogenum* produce dhilirolides A−D (**1**−**4**), four meroterpenoids representing the unprecedented dhilirane (**I**) and isodhilirane (**II**) carbon skeletons (Figure 1).[14] Continued chemical investigation of laboratory cultures of *P. purpurogenum* has resulted in the isolation of the ten new meroterpenoids dhilirolides E−N (**5**−**14**), which have revealed the additional unprecedented 14,15-dinordhilarane (**III**) and 23,24-dinorisodhilarane (**IV**) carbon skeletons (Figure 1). The dhilirolides were found to have promising feeding inhibition and developmental disruption activities against the cabbage looper *Trichoplusia ni* (Lepidoptera: Noctuidae), an economically important agricultural pest. Details of the isolation and structure elucidation of the new dhilirolides E−N (**5**−**14**), a stable isotope feeding study that has provided insight into the biogenesis of the new carbon skeletons, and the insecticidal activities of the dhilirolides are presented here.

RESULTS AND DISCUSSION

Laboratory cultures of *P. purpurogenum* were grown on the surface of potato dextrose agar, and the agar and attached fungal mycelium were then extracted with EtOAc as previously described.[14] The residue obtained by evaporation of the combined EtOAc extracts was fractionated by sequential application of Sephadex LH20 chromatography and C_{18} and C_8 reversed-phase HPLC to give pure samples of dhilirolides A−N (**1**−**14**).

Dhilirolide E (**5**) was isolated as an optically active amorphous white powder that gave a $[M + Na]^+$ ion in the HRESITOFMS at m/z 465.1887 appropriate for a molecular formula of $C_{25}H_{30}O_7$, which was identical to that of dhilirolide

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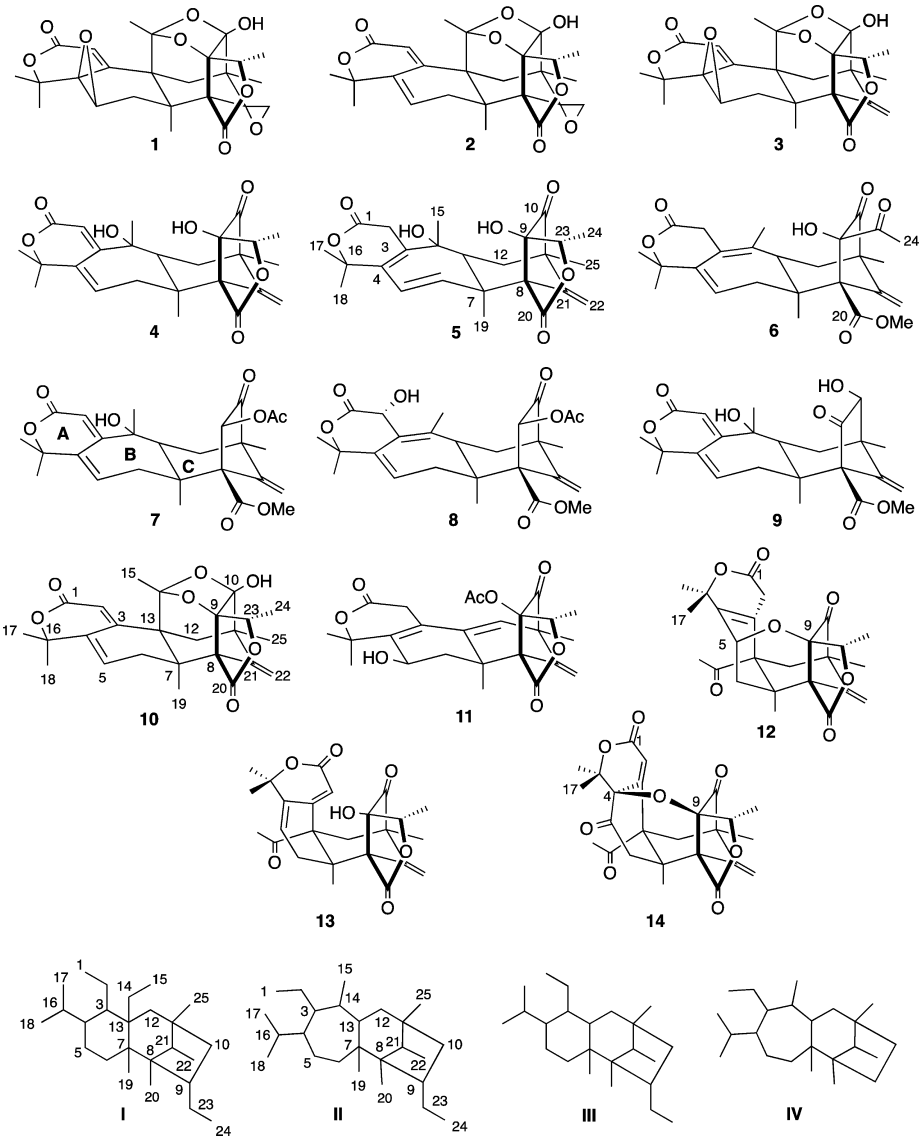

Figure 1. Structures of dhilirolides A (**1**)−N (**14**) and the dhilirane (**I**), isodhilirane (**II**), 14,15-dinordhilarane (**III**), and 23,24-dinorisodhilarane (**IV**) meroterpenoid carbon skeletons.

D (**4**). Although the ^{1}H and ^{13}C NMR spectra of **5** were similar to those of **4**, their UV spectra were markedly different. In dhilirolide D (**4**), a λ_{max} typical of a dienone was observed at 276 nm, while in **5** the λ_{max} was reduced to 248 nm indicating a break in conjugation with the lactone but still indicative of a diene. Simply shifting the 2,4-diene present in **4** to a 3,5-diene in **5** would explain the hypsochromic shift in the λ_{max}. Examination of the ^{1}H/^{13}C/gCOSY/gHSQC/gHMBC NMR data (Tables 1 and 2 and Supporting Information) obtained for **5** revealed that the olefinic H-2 resonance (δ 6.06) in **4** had been replaced by diastereotopic methylene proton resonances at δ 3.29 and 3.45, which showed gHMBC correlations to the lactone carbonyl resonance assigned to C-1 (δ 170.6). The NMR data of **5** identified the $\Delta^{21,22}$ exocyclic alkene seen in **4** and also revealed the presence of additional tetrasubstituted (C-3: δ 136.4; C-4: δ 129.4) and disubstituted [C-5: δ 118.1, H-5: δ 5.62 (d, J = 12.3 Hz); C-6: δ 143.3, H-6: δ 5.98 (d, J = 12.3 Hz)] olefins. gHMBC correlations observed between H-2α/β (δ 3.45/3.29) and C-3 (δ 136.4) and C-4 (δ 129.4), between H-5 (δ 5.62) and C-3 (δ 136.4), between H-6 (δ 5.98) and C-4 (δ 129.4), between Me-15 (δ 1.08) and C-3 (δ 136.4), between Me-17/Me-18 (δ 1.47/1.38) and C-4 (δ 129.4), and between Me-19 (δ 1.29) and C-6 (δ 143.3) identified a 3,5-diene substructure in **5**. tROESY correlations confirmed that the relative configuration of **5** was the same as **4**, and, therefore, the absolute configuration of dhilirolide E (**5**) has been assigned as 7*S*,8*R*,9*R*,11*R*,13*R*,14*R*,23*S*.

Dhilirolide F (**6**) was isolated as an optically active solid that gave a [M + Na]$^{+}$ ion in the HRESITOFMS at m/z 479.2050 appropriate for a molecular formula of $C_{26}H_{32}O_7$, which differs from the molecular formula of dhilirolides D (**4**) and E (**5**) by the addition of a carbon and two protons but requires the same 11 sites of unsaturation. A UV λ_{max} at 240 nm suggested a diene in the structure of **6** as is found in **5**. However, significant differences in the ^{1}H and ^{13}C NMR spectra of dhilirolides F (**6**) and E (**5**) (Tables 1 and 2 and Supporting Information) showed that the diene in **6** was in a different location than the diene in **5**. The NMR data for **6** identified a trisubstituted $\Delta^{4,5}$ alkene (C-4: δ 136.8; C-5: δ 129.1, H-5: δ 6.15, dd, J = 8.7, 4.1 Hz), which is also found in dhilirolide D (**4**), but the resonance assigned to the OH-14 proton in dhilirolide D (**4**) and E (**5**) was missing and Me-15 had a chemical shift at δ 1.60 (s) (C-15:

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Table 1. ^{1}H NMR Data for Dhilirolides E–I (5–9) Recorded in DMSO-d_6

	δ_H (J, Hz)				
position no.	E (**5**)	F (**6**)	G (**7**)	H (**8**)	I (**9**)
2α	3.45 d (19.9)	3.28 d (21.5)	6.05 s	4.79 d (5.1)	6.11 s
2β	3.29 d (19.9)	3.49 d (21.5)			
5	5.62 d (12.3)	6.15 dd (8.7, 4.1)	6.04 bm	6.18 m	6.03 dd (7.2, 3.9)
6α	5.98 d (12.3)	2.20 dd (14.3, 8.7)	2.21 dd (19.5, 7.2)	1.83 dd (14.9, 4.6)	2.63 dd, (15.9, 7.2)
6β		1.62 dd (14.3, 4.1)	3.03 dd (19.5, 2.6)	2.07 dd (14.9, 8.2)	2.94 dd (15.9, 3.9)
9			5.78 s	5.81 s	
10					3.62 d (5.1)
12$_{ax}$	2.24 t (13.1)	1.85 t (12.8)	1.71 t (11.9)	1.93 dd (12.3, 12.3)	1.78 t (12.8)
12$_{eq}$	1.71 dd (13.1, 5.2)	1.56 dd (12.8, 4.9)	1.93 dd (11.9, 4.4)	1.60 dd (12.3, 4.6)	1.92 dd (12.8, 5.1)
13	2.51 dd (13.1, 5.2)	2.75 dd (12.8, 4.9)	2.27 dd (11.9, 4.4)	2.49 nd[a]	1.86 dd (12.8, 5.1)
15	1.08 s	1.60 s	1.23 s	1.80 s	1.18 s
17	1.47 s	1.31 s	1.40 s	1.30 s	1.53 s
18	1.38 s	1.50 s	1.54 s	1.57 s	1.38 s
19	1.29 s	1.10 s	1.11 s	1.26 s	1.11 s
22α	5.19 s	5.05 s	5.13 s	5.14 s	5.13 s
22β	4.97 s	5.61 s	5.25 s	5.23 s	4.70 s
23	4.50 q (7.0)				
24	1.04 d (7.0)	2.17 s			
25	1.14 s	1.06 s	1.13 s	1.12 s	1.21 s
2-O*H*				5.90 d (5.1)	
9-O*H*	6.88 s	6.78 s			
9-O*Ac*			2.18 s	2.26 s	
10-O*H*					6.19 d, (5.1)
14-O*H*	4.78 s		5.32 s		5.26 bs
20-O*Me*		3.60 s	3.71 s	3.71 s	3.58 s

[a]Multiplicity not determined due to overlapping signals/chemical shifts determined from 2D data.

δ 15.0) appropriate for an olefinic methyl. A $\Delta^{3,14}$ tetrasubstituted alkene (C-3: δ 125.6; C-14: δ 135.5) satisfied these spectroscopic observations, and gHMBC correlations between H-2α/β (δ 3.28/3.49) and C-3 (δ 125.6), C-4 (δ 136.8) and C-14 (δ 135.5), between H-5 (δ 6.15) and C-3 (δ 125.6), between H-13 (δ 2.75) and C-3 (δ 125.6), C-14 (δ 135.5) and C-15 (δ 15.0), and between Me-15 (δ 1.60) and C-3 (δ 125.6), C-13 (δ 41.4) and C-14 (δ 135.5) confirmed the $\Delta^{3,14}$ structural feature. Although the characteristic oxymethine quartet assigned to H-23 (δ 4.51- 4.89) in dhilirolides D (**4**) and E (**5**) was missing in the ^{1}H NMR spectrum of **6**, a singlet resonance at δ 6.78, which did not show a gHSQC correlation to a carbon atom, could still be assigned to a tertiary alcohol at C-9. The OH-9 resonance showed gHMBC correlations not only to the C-10 (δ 212.9) ketone, C-8 (δ 67.9), and C-9 (δ 92.4) but also to a second ketone carbon resonance at δ 207.0 (C-23). A methyl singlet with a chemical shift (δ 2.17, Me-24) appropriate for a α-keto methyl also showed HMBC correlations to the ketone resonance at δ 207.0 (C-23) and the C-9 resonance at δ 92.4, establishing the presence of a methyl ketone substitutent at C-9. The remaining degree of unsaturation and $C_2H_3O_2$ fragment required by the molecular formula was attributed to a methoxy carbonyl substituent at C-8 in **6** in place of the lactone carbonyl found at C-8 in **4** and **5**. A strong gHMBC correlation observed between a methyl singlet at δ 3.60 and an ester carbonyl at δ 168.9, assigned to C-20, supported a methoxy carbonyl substituent at C-8. tROESY correlations observed between the OH-9 (δ 6.78) resonance and the H-5 (δ 6.15) and H-13 (δ 2.75) resonances showed that the configuration at C-9 was identical to the C-9 configuration in dhilirolide D (**4**) and additional tROESY correlations revealed that the relative configurations at the C-7, C-8, C-11, and C-13 stereogenic centers shared by **4** and **6** were identical, leading to the absolute configuration assignment of 7*S*,8*R*,9*R*,11*R*,13*R* for dhilirolide F (**6**).

Dhilirolide G (**7**) was isolated as an optically active solid that gave a $[M + Na]^+$ ion in the HRESITOFMS at m/z 495.1982 appropriate for a molecular formula of $C_{26}H_{32}O_8$, which differs from the molecular formula of dhilirolide F (**6**) by the addition of an oxygen. Detailed analysis of the 1D and 2D NMR data (Tables 1 and 2 and Supporting Information) and UV spectra (λ_{max} 269 nm) obtained for dhilirolide G (**7**) revealed that the constitution of rings A, B, and C were the same as in dhilirolide D (**4**) and that the methyl ester seen in dhilirolide F (**6**) at C-20 [C-20: δ 169.9; 20-OMe: δ_C 51.7, δ_H 3.71, s] was intact. However, compared with **6**, the resonances assigned to the C-23/C-24 methyl ketone substituent attached at C-9 and the OH-9 at C-9 had been replaced by resonances appropriate for an acetylated oxymethine [C-9: δ 78.4, H-9: δ 5.78; OAc: δ_C 168.4, 20.4, δ_H 2.18]. gHMBC correlations between the H-9 oxymethine singlet resonating at δ 5.78 and the acetate carbonyl at δ 168.4, and between the H-9 resonance and the C-7 (δ 44.3), C-8 (δ 64.7), C-10 (δ 210.3), C-11 (δ 52.4: weak), and C-20 (δ 169.9) resonances, confirmed the structural fragment and accounted for the molecular formula. tROESY correlations between the H-9 (δ 5.78) and both the H-6β (δ 3.03) and H-13 (δ 2.27) resonances showed that H-9 was *cis* to C-7, and therefore, the absolute configuration for **7** was assigned as 7*S*, 8*R*, 9*R*, 11*R*, 13*R*, and 14*R*.

Dhilirolide H (**8**) was isolated as an optically active solid that gave a $[M - H]^-$ ion in the HRESITOFMS at m/z 471.2014 appropriate for a molecular formula of $C_{26}H_{30}O_8$, the same as dhilirolide G (**7**). Extensive analysis of the 1D and 2D NMR spectra of dhilirolide H (**8**) (Tables 1 and 2 and Supporting

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Table 2. ^{13}C NMR Data for Dhilirolides E−G (5−14) Recorded in DMSO-d_6

	δ_C									
position no.	E (5)	F (6)	G (7)	H (8)[a]	I (9)	J (10)	K (11)[a]	L (12)	M (13)	N (14)[a]
1	170.6	169.6	163.7	170.8	164.0	162.8	169.5	167.6	163.3	161.1
2	32.7	34.1	113.9	65.5	130.2	114.8	30.6	30.8	114.3	128.6
3	136.4	125.6	161.7	130.5	163.7	150.3	122.0	128.7	147.1	151.4
4	129.4	136.8	133.5	138.9	130.0	133.3	139.3	135.7	130.6	77.5
5	118.1	129.1	129.6	127.5	130.1	130.1	63.5	64.8	129.4	196.5
6	143.3	35.9	38.9	35.9	37.0	35.1	40.5	33.3	37.3	43.3
7	44.9	61.0	44.3	60.3	46.8	40.0	42.4	37.2	41.1	40.7
8	65.8	67.9	64.7	65.7	70.5	59.4	63.2	60.3	67.6	65.2
9	89.0	92.4	78.4	78.6	212.6	91.8	94.0	87.0	88.1	91.3
10	213.9	212.9	210.3	211.7	80.4	108.6	197.1	212.6	211.9	211.2
11	54.9	53.1	52.4	52.5	45.4	49.1	55.6	53.9	54.9	55.4
12	42.5	41.7	40.4	39.5	33.8	38.3	122.1	44.6	39.5	48.8
13	52.9	41.4	52.4	42.3	48.8	47.4	143.1	59.0	59.9	57.4
14	74.6	135.5	74.1	141.2	75.4	107.3		204.5	202.7	209.3
15	29.3	15.0	33.2	15.5	32.5	20.3		28.2	26.3	30.7
16	83.7	81.9	82.8	83.0	82.5	82.0	84.0	83.7	81.2	81.9
17	26.5	28.4	26.9	30.1	25.9	29.4	29.0	25.6	26.4	22.5
18	26.7	26.0	26.2	28.0	26.3	27.7	25.4	26.2	28.2	25.4
19	11.5	22.4	16.8	22.2	14.8	19.7	21.4	17.2	23.9	19.5
20	173.2	168.9	169.9	169.6	168.7	171.5	170.7	171.2	172.7	172.0
21	148.0	147.2	145.6	146.4	149.7	150.1	144.0	147.4	148.6	144.9
22	107.1	106.7	108.4	107.6	106.4	106.4	110.0	106.9	107.8	109.8
23	82.8	207.0				80.9	77.9	80.8	83.6	82.0
24	17.9	26.8				18.5	18.6	16.5	17.6	17.4
25	15.4	16.8	15.7	15.8	20.7	15.7	13.2	15.4	15.8	15.2
20-O*Me*		51.2	51.7	52.3	52.0					
9-O*Ac*			168.4/20.4	169.2/20.7			170.2/21.7			

[a]Many of the chemical shift assignments are based on interpretation of the 2D NMR data due to limited material.

Information) and comparison with the NMR data for dhilirolides D−G (**4**−**7**) revealed that the constitution of ring B was the same as that seen in dhilirolide F (**6**), and rings C and D were the same as seen in dhilirolide G (**7**), while structural novelty was present in ring A. An oxymethine doublet resonating at δ 4.79 (J = 5.1 Hz: H-2) was coupled in the gCOSY60 experiment to a hydroxyl doublet at δ 5.90 (J = 5.1 Hz). gHMBC correlations between H-2 (δ 4.79) and C-1 (δ 170.8), C-3 (δ 130.5), C-4 (δ 138.9), and C-14 (δ 141.2) placed a secondary alcohol at C-2. The observation of tROESY correlations between Me-17 (δ 1.30) and both of the H-2 (δ 4.79) and H-13 (δ 2.49) resonances required the alcohol at C-2 to be in the α orientation as shown. Additional tROESY correlations observed between H-9 (δ 5.81) and both H-6β (δ 2.07) and H-13 (δ 2.49) showed that the relative configuration at C-9 in **8** was the same as in **7**, in agreement with the nearly identical ^{13}C chemical shifts observed for C-8 to C-12 in **7** and **8** (Table 2). Two quite different conformations, which must be equilibrating rapidly on the NMR time scale, are required to generate the observed tROESY correlations between Me-17 and H-2 and between Me-17 and H-13 in **8**. The calculated lowest potential energy conformations of **7** and **8**, which confirm the proximity of H-9 and both H-6β and H-13, are given in the Supporting Information. By analogy with dhilirolide D (**4**), the absolute configuration of dhilirolide H (**8**) was assigned as 2*R*,7*S*,8*R*,9*R*,11*R*,13*R*.

Dhilirolide I (**9**) gave a $[M + Na]^+$ ion in the HRESITOFMS at m/z 453.1920 appropriate for the molecular formula $C_{24}H_{30}O_7$, which differs from that of dhilirolide D (**4**) by the loss of one carbon atom and required only 10 sites of unsaturation instead of 11. Examination of the ^{1}H and ^{13}C NMR spectra recorded for dhilirolide I (**9**) (Tables 1 and 2 and Supporting Information) revealed a close relationship with dhilirolides D (**4**) and G (**7**) but also several significant differences in ring D. Resonances attributable to the methyl esters seen in **6**, **7**, and **8** were observed in the NMR data obtained for **9** (C-20: δ 168.7; 20-OMe: δ_C 52.0, δ_H 3.58). However, the methyl singlet assigned to Me-25 (δ 1.21) in **9** no longer showed a gHMBC correlation to a ketone resonance that could be assigned to C-10. Instead a correlation was seen to a carbinol methine resonating at δ 80.4, that in the gHSQC correlated to a doublet at δ 3.62 (J = 5.1 Hz) (H-10), which was in turn coupled in the gCOSY60 to an exchangeable resonance at δ 6.19 (d, J = 5.1 Hz) (OH-10). These observations suggested a switch in the locations of the C-9 carbinol methine and C-10 ketone seen in dhilirolides G (**7**) and H (**8**) to a C-9 ketone and C-10 carbinol methine in dhilirolide I (**9**). gHMBC correlations between the H-10 resonance (δ 3.62) and a ketone resonance at δ 212.6, that could only be assigned to C-9 since H-10 also correlated with C-11 (δ 45.4), C-12 (δ 33.8), and C-25 (δ 20.7) confirmed the switch. The change in functionality at C-9 and C-10 in **9** was further supported by a tROESY correlation between H-10 (δ 3.62) and Me-25 (δ 1.21) and a strong gHMBC correlation between Me-25 (δ 1.21) and C-10 (δ 80.4). tROESY correlations observed between OH-10 (δ 6.19) and both H-12$_{eq}$ (δ 1.92) and H-13 (δ 1.86), and between H-10 (δ 3.62) and both of the H-22 resonances (δ 4.70, 5.13) showed that OH-10 was *cis* to C-12. Therefore, the absolute configuration of dhilirolide I (**9**) was assigned as 7*S*,8*R*,10*R*,11*R*,13*R*,14*R*.

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Table 3. ^{1}H NMR Data for Dhilirolides J–N (10–14) Recorded in DMSO-d_6

	δ_H (J, Hz)				
position no.	J (**10**)	K (**11**)	L (**12**)	M (**13**)	N (**14**)
2α	5.77 s	3.06 bd (19.9)	2.58 d (19.8)	5.94 s	6.80 s
2β		3.43 dd (19.9, 2.3)	2.90 d (19.8)		
5	6.21 m	4.39 bm	4.77 bs	5.97 bm	
6α	1.98 dd (19.2, 6.1)	2.04 dd (11.5, 5.5)	2.19 bd(13.6)[a]	2.48 dd (20.6, 4.1)	2.81 d(20.7)[a]
6β	3.00 d (19.2)	1.70 bt (11.5)	1.80 bd (13.6)[a]	2.61 dd (20.6, 3.8)	2.79 d (20.7)[a]
12_{ax}	1.45 d (12.3)	5.35 s	2.55 d (14.1)	2.38 d (14.3)	3.06 d (13.6)
12_{eq}	2.09 d (12.3)		1.87 d (14.1)	3.00 d (14.3)	2.91 d (13.6)
15	1.48 s		2.06 s	2.05 s	2.36 s
17	1.50 s	1.52 s	1.55 s	1.49 s	1.55 s
18	1.54 s	1.63 s	1.49 s	1.12 s	1.36 s
19	0.95 s	1.34 s	1.27 s	1.41 s	1.40 s
22α	5.07 s	5.38 s	5.25 bs	5.19 s	5.36 s
22β	4.93 s	5.08 s	5.03 bs	4.98 bs	5.16 s
23	4.68 q (7.0)	4.92 q (7.0)	4.45 q (7.2)	4.32 q (7.1)	4.69 q (7.7)
24	1.37 d (7.0)	1.10 d (7.0)	1.00 d (7.2)	0.95 d (7.1)	0.97 d (7.7)
25	1.10 s	1.35 s	1.14 s	1.24 s	1.21 s
5-O*H*		5.24 d (6.2)			
9-O*H*				6.57 s	
9-O*Ac*		2.07 s			
10-O*H*	7.27 s				

[a]Assignments within a column maybe interchanged.

Dhilirolide J (**10**) was isolated as an optically active crystalline solid that gave a $[M + H]^+$ ion in the HRESITOFMS at m/z 441.1918 appropriate for a molecular formula of $C_{25}H_{28}O_7$, which differed from dhilirolides B (**2**) and C (**3**) by the loss of one oxygen atom. Comparison of the ^{1}H and ^{13}C NMR spectrum of dhilirolide J (**10**) (Tables 2 and 3 and Supporting Information) with the corresponding spectra recorded for dhilirolides B (**2**) and C (**3**) revealed the absence of resonances that could be attributed to epoxide fragments. Analysis of the NMR data and UV spectra (λ_{max} 279 nm) suggested that dhilirolide J (**10**) contained the C-1 to C-5 dienone (C-1: δ 162.8; C-2: δ 114.8, H-2: δ 5.77; C-3: δ 150.3; C-4: δ 133.3; C-5: δ 130.1, H-5: δ 6.21) seen in dhilirolide B (**2**). As with dhilirolide C (**3**), the C-21/C-22 epoxide found in dhilirolide A (**1**) had been replaced with a $\Delta^{21,22}$ exocyclic alkene (C-21: δ 150.1; C-22: δ 106.4, H-22α/β: δ 5.07/4.93) in **10**. The stereogenic centers in **10** are all shared with dhilirolide A (**1**), and therefore, the absolute configuration of **10** was assigned as 7*R*,8*R*,9*R*,10*R*,11*R*,13*S*,14*S*,23*S*.

Dhilirolide K (**11**) gave a $[M + Na]^+$ ion in the HRESITOFMS at m/z 479.1679 appropriate for a molecular formula of $C_{25}H_{28}O_8$. Although this was the same molecular formula as observed for dhilirolide C (**3**), inspection of the NMR data (Tables 2 and 3 and Supporting Information) and UV spectra for **11** revealed significant structural and functional group differences compared with **3**. Reminiscent of dhilirolides D (**4**) and E (**5**), NMR resonances observed for **11** could be assigned to the γ-lactone and ring D substructures seen in **4** and **5** with the one difference being that an acetate functionality (δ_C 170.2/21.7, δ_H 2.07) was at C-9 in **11** in place of an exchangeable OH-9 proton. The UV λ_{max} at 249 nm for **11** was suggestive of a diene and not the enone that is sometimes encountered in ring A of the dhilirolides. A pair of diastereotopic methylene resonances at δ 3.06 and 3.43 (H-2α/β) in the ^{1}H NMR spectrum of **11**, that showed gHMBC correlations to the lactone carbonyl assigned to C-1 (δ 169.5), supported the absence of a $\Delta^{2,3}$ alkene in **11**. However, the NMR data for **11** did confirm the presence of tetra- and trisubstituted conjugated $\Delta^{3,4}$ and $\Delta^{12,13}$ olefins (C-3: δ 122.0; C-4: δ 139.3; C-12: δ 122.1, H-12: δ 5.35; C-13: δ 143.1). A proton resonance at δ 5.24 (d, J = 6.2 Hz), which did not show a gHSQC correlation to a carbon atom but coupled to a multiplet at δ 4.39 (H-5) in the gCOSY60, was assigned to a secondary alcohol at C-5. The OH-5 showed gHMBC correlations to one of the olefinic carbons of the diene at δ 139.3. Since Me-17/18 (δ 1.52/1.63) and H-2α/β(δ 3.06/3.43) also correlated to the carbon at δ 139.3 in the gHMBC, this resonance was assigned to C-4. These observations supported tetrasubstituted $\Delta^{3,4}$ and trisubstituted $\Delta^{12,13}$ conjugated alkenes in the structure of **11** and the absence of the C-14-C-15 carbon fragment that is common to dhilirolides A–C (**1**–**3**) and J (**10**). The addition of the acetate at C-9 balances the loss of the C_2H_3O C-14/C-15 fragment and satisfies the constraints imposed by the molecular formula. gHMBC correlations observed between Me-19 (δ 1.34) and C-13 (δ 143.1), between Me-25 (δ 1.35) and C-12 (δ 122.1), between H-12 (δ 5.35) and both C-3 (δ 122.0) and C-13 (δ 143.1), and between H-2α/β (δ 3.06/3.43) and C-3 (δ 122.0), C-4 (δ 139.3), and C-13 (δ 143.1) were consistent with the proposed structure **11**. The tROESY data for dhilirolide K (**11**) showed correlations between H-5 (δ 4.39) and both Me-18 (δ 1.63) and Me-19 (δ 1.34) suggesting that the C-5 alcohol is in a β orientation. Based on the assumption that the configurations at the common stereogenic centers in dhilirolides A (**1**) and **11** are the same, a detailed analysis of the 2D tROESY data obtained for **11** confirmed that the absolute configuration of **11** is 5*R*,7*S*,8*R*,9*R*,11*S*,23*S*.

Dhilirolide L (**12**) was isolated as optically active clear prism like crystals that gave a $[M + Na]^+$ ion in the HRESITOFMS at m/z 463.1745 appropriate for a molecular formula of $C_{25}H_{28}O_7$. This was the same molecular formula as observed for dhilirolide J (**10**), but there were significant differences in the NMR data obtained for **10** and **12** (Tables 2 and 3 and Supporting Information). As with dhilirolide K (**11**), the γ-

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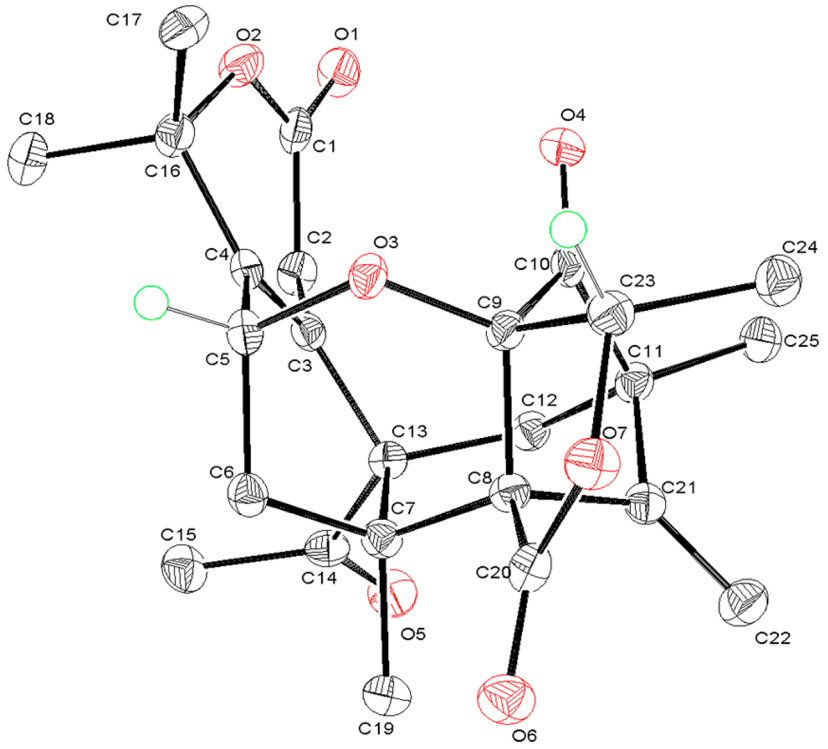

Figure 2. ORTEP diagram for dhilirolide L (**12**).

lactone and ring D substructures seen in **4** and **5** were present in the structure of **12**. However, instead of an acetate substituent at C-9 an ether linkage between C-5 and C-9 was revealed by a gHMBC correlation observed between H-5 (δ 4.77) and C-9 (δ 87.0). The lack of any significant UV absorption for **12** precluded any of the conjugation seen in dhilirolides A–K (**1**–**11**). gHMBC correlations between the diastereotopic methylene proton resonances assigned to H-2α/β (δ 2.58/2.90) and C-3 (δ 128.7) and C-4 (δ 135.7), between H-5 (δ 4.77) and C-3 (δ 128.7) and C-4 (δ 135.7), and between Me-17/18 (δ 1.55/1.49) and C-4 (δ 135.7) established the presence of a tetrasubstituted $\Delta^{3,4}$ alkene. gHMBC correlations observed between a methyl singlet at δ 2.06, assigned to Me-15 and carbon resonances at δ 204.5 (C-14), assigned to a ketone, and a quaternary resonance at δ 59.0, assigned to C-13, were consistent with a methyl ketone substituent attached to C-13.

The rigidity of dhilirolide L (**12**), resulting from its fused polycyclic structure, facilitated the assignment of the relative configuration after analysis of all of the observed tROESY data. However, there was no unambiguous NMR evidence for the configuration at C-5, even though the proposed caged structure required that C-5 had the same configuration as in **11**. In order to verify the proposed structure, dhilirolide L (**12**) was subjected to single crystal X-ray diffraction analysis, using Cu Kα radiation. The absolute configuration was established on the basis of the refined Flack *x*-parameter [x = 0.00(4)].[15,16] The ORTEP diagram in Figure 2 shows the constitution and absolute configuration (5*R*,7*S*,8*R*,9*R*,11*R*,13*R*,23*S*) of dhilirolide L (**12**). At the common stereogenic centers, this is identical to the absolute configuration determined by single-crystal X-ray diffraction analysis for dhilirolide A (**1**)[14] except at C-13, where the configuration in **12** has been inverted compared with **1**.

Dhilirolide M (**13**) gave a $[M + Na]^+$ ion in the HRESITOFMS at m/z 463.1740 appropriate for a molecular formula of $C_{25}H_{28}O_7$, the same as dhilirolide L (**12**). Although the ^{1}H and ^{13}C NMR spectrum of **13** were similar to that of **12** (Tables 2 and 3), the UV spectrum was markedly different. A λ_{max} at 273 nm was again indicative of the dienone substructure seen in dhilirolides B (**2**), D (**4**), G (**7**), I (**9**), and J (**10**). Examination of the NMR data for **13** (Table 2 and 3 and Supporting Information) confirmed the presence of a C-1 to C-5 dienone. Since all other structural features of **13** appeared to be the same as in dhilirolide L (**12**), it was apparent that a tertiary alcohol should reside at C-9 to satisfy the requirements of the molecular formula. A proton resonance at δ 6.57 (s), that did not show a gHSQC correlation to a carbon, was assigned to the alcohol at C-9. The OH-9 showed gHMBC correlations to the ketone resonance at δ 211.9, assigned to C-10, a quaternary resonance at δ 67.6, assigned to C-8, an oxygenated methine resonance at δ 83.6, assigned to C-23, and a tertiary carbinol resonance at δ 88.1, assigned to C-9 which were consistent with the proposed structure. The tROESY data was consistent with the assumption that the configurations at all the common stereogenic centers in **12** and **13** were the same giving the absolute configuration of **13** as 7*S*,8*R*,9*R*,11*R*,13*R*,23*S*.

Dhilirolide M (**13**) was isolated as a single peak from reversed-phase HPLC that was well resolved from the HPLC peak containing dhilirolide L (**12**), as described in the Experimental Section. However, when the 1D ^{1}H NMR spectrum of dhilirolide M (**13**) was recorded in DMSO-d_6, it showed evidence for small amounts of dhilirolide L (**12**). During the course of collecting a 2D NMR data set on dhilirolide M (**13**), the amount of dhilirolide L (**12**) in the sample increased until it was present at roughly 10% of the dhilirolide M (**13**) concentration. Longer exposure in the NMR tube in DMSO-d_6 resulted in almost complete conversion of dhilirolide M (**13**) to dhilirolide L (**12**). It was also observed

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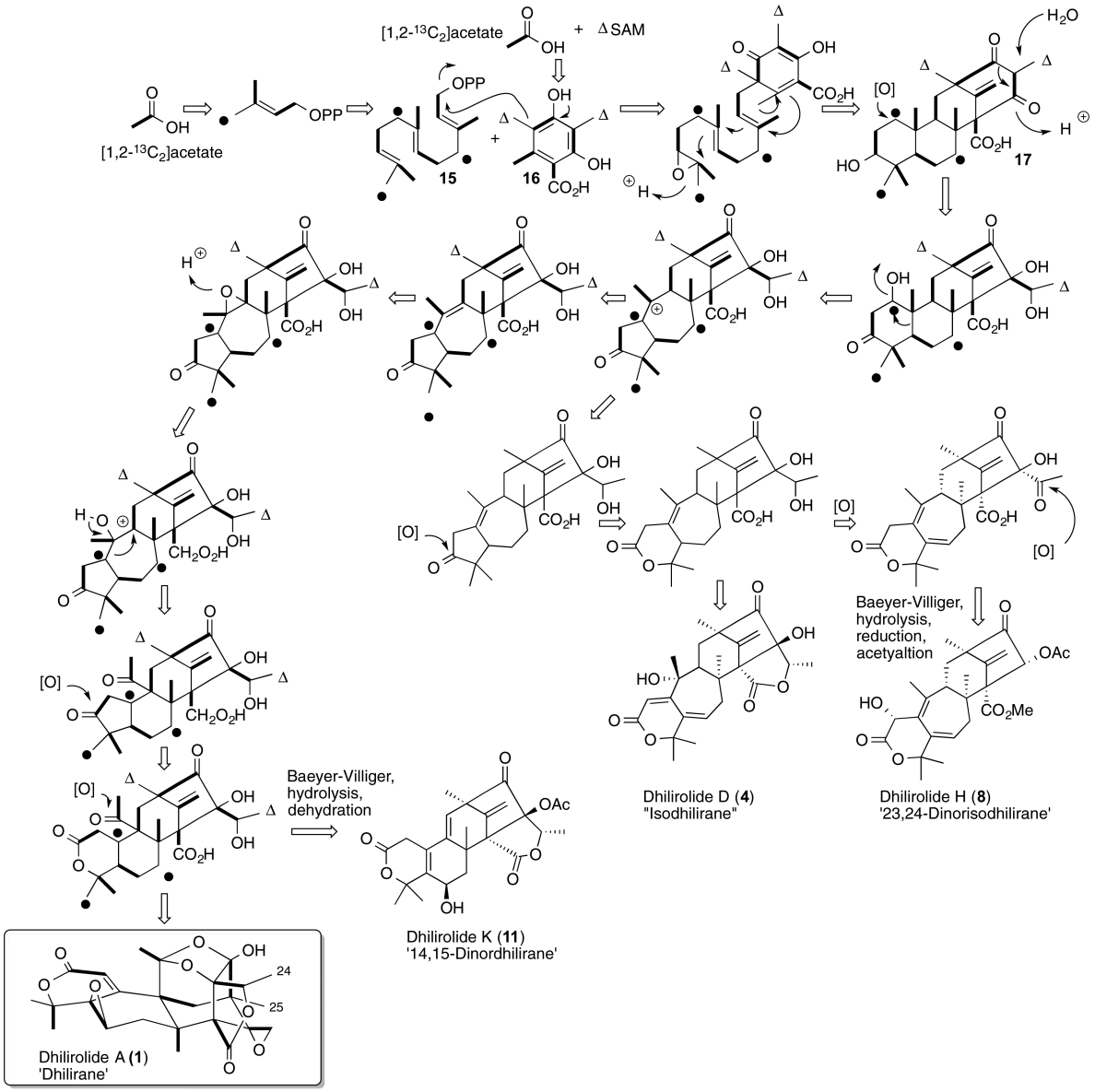

Figure 3. Proposed biogenetic pathway for the dhilirolides showing representative examples of each of the new dhilirane, isodhilirane, 14,15-dinordhilirane, and 23,24-dinorisodhilirane skeletons. The labeling pattern indicating intact acetate units shown for dhilirolide A (**1**) was determined from the [1,2-$^{13}C_2$]acetate feeding study and an INADEQUATE experiment (see the Supporting Information) and is consistent with the proposed biogenesis as illustrated.

that NMR samples of dhilirolide L (**12**) that sat for several days in DMSO-d_6 started to show the presence of minor amounts of dhilirolide M (**13**). Thus, it appears that dhilirolides L (**12**) and M (**13**) are able to slowly interconvert in DMSO-d_6.

Dhilirolide N (**14**) gave a $[M + Na]^+$ peak in the HRESITOFMS at m/z 477.1521 appropriate for a molecular formula of $C_{25}H_{26}O_8$, which differs from the molecular formula for dhilirolide L (**12**) by the loss of two protons, the addition of one oxygen, and requires one additional site of unsaturation. Comparison of the NMR data obtained for **12** and **14** (Table 2 and 3 and Supporting Information) revealed the presence of an additional ketone resonance at δ 196.5 (C-5) that accounted for the additional site of unsaturation, the extra oxygen, and the loss of two hydrogen atoms. The third ketone in **14** was situated at C-5 since gHMBC correlations were observed between C-5 (δ 196.5) and both H-6α/β (δ 2.81/2.79) and Me-19 (weak, δ 1.40). A singlet olefinic methine resonance at δ 6.80 (H-2) was suggestive of the trisubstituted α,β-unsaturated lactone seen in dhilirolides A (**1**) and C (**3**). gHMBC correlations from H-2 (δ 6.80) to the lactone C-1 carbonyl (δ 161.1) and C-13 (δ 57.4) and between H-12$_{ax}$ (δ 3.06) and C-3 (δ 151.4) confirmed the presence of an enone in ring A. An oxygenated tertiary carbon resonating at δ 77.5 was assigned to C-4 since it showed gHMBC correlations to both H-2 (δ 6.80) and Me-17/18 (δ 1.55/1.36). The γ-lactone and ring D substructures and the methyl ketone residue at C-13 in **14** were all identical to those seen in dhilirolide L (**12**). With no other apparent structural or functional differences between **12** and **14** simply satisfying the valences on the two oxygenated quaternary carbons C-4 and C-9 (δ 77.5 and 91.3, respectively), through an ether linkage provided the final ring required by the molecular formula and completed the constitution of **14**. A tROESY correlation observed between Me-17 (δ 1.55) and H-23 (δ 4.69) provided additional support for the C-4/C-9 ether

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linkage and established the relative configuration at C-4. By analogy with dhilirolide L (**12**), the absolute configuration of **14** was assigned as 4*R*,7*S*,8*R*,9*R*,11*R*,13*R*,23*S*.

The dhilirolides are presumed to be highly rearranged meroterpenoids.[14] Dhilirolides D−I (**4**−**9**), possessing the isodhilirane (**II**) or 23,24-dinorisodhilirane (**IV**) carbon skeletons, share many structural features with berkeleydione[17] and the miniolutelides.[18] The biogenetic origin of these two metabolites is thought to be from a mixed terpenoid/polyketide biogenesis involving farnesyl pyrophosphate (**15**) and 3,5-dimethylorsellinate (**16**)[19] as demonstrated for the 25 carbons in the fungal metabolites terretonin,[20] citreohybridines[21] and recently austinol.[22] With this in mind, we undertook a stable isotope feeding study with [1,2-$^{13}C_2$]acetate aimed at providing experimental support for the proposed biogenesis of the new dhilirane (**I**), isodhilirane (**II**), 14,15-dinordhilarane (**III**), and 23,24-dinorisodhilarane (**IV**) carbon skeletons shown in Figure 2. In the [1,2-$^{13}C_2$]acetate feeding study, no statistically significant ^{13}C-enrichment was observed at any site, just as was found in the terretonin[20] study when labeled [1-^{13}C]-acetate was fed. However, ^{13}C−^{13}C coupling satellites were observed for all the carbon resonances except C-3, C-6, C-17, C-24, and C-25 in the ^{13}C spectra of the dhilirolides obtained from *P. purpurogenum* cultures fed [1,2-$^{13}C_2$]acetate. The average level of enrichment was estimated at 0.14% from the relative heights of the coupling satellites and the singlet natural abundance signals. Dhilirolide A (**1**) was isolated in sufficient quantities to acquire a 2D-INADEQUATE spectrum, which allowed the unambiguous assignment of intact acetate units as summarized in Figure 3.

A proposed biogenetic pathway to the dhilirolides that accounts for the [1,2-$^{13}C_2$]acetate incorporation data is shown in Figure 3. The pathway provides additional support for the earlier labeling work of the Simpson and Vederas groups.[19,20,23] As observed, no incorporation would be expected in the dhilirolides at Me-24 and Me-25 since the 3- and 5-methyl substituents of dimethylorsellinate (**16**) are presumably derived from SAM.[20,22] Recent targeted gene deletion studies have shown that two different gene clusters on two different chromosomes are required to make the meroterpenoids dehydroaustinol and austinol in *Aspergillus nidulans*.[22] By analogy, it seems likely that one gene cluster in *P. purpurogenum* contains a PKS gene for the production of 3,5-dimethylorsellinate (**16**), and a second cluster contains the prenyltransferase gene for the C alkylation of the dimethylorsellinic acid (**16**). Subsequent cyclization would give the tetracyclic intermediate **17** which would then undergo significant modification to form the dhilirolides.

The crude fungal extract as well as the major constituents dhilirolides A (**1**), D (**4**), and L (**14**) were evaluated for bioactivity against the cabbage looper *Trichoplusia ni* (Lepidoptera: Noctuidae), an economically important agricultural pest. The crude extract was a modest feeding inhibitor to third instar *T. ni* larvae, but an LH20 fraction enriched in dhilirolides A−N (**1**−**14**) was considerably more active in a two-choice feeding bioassay. The DC_{50} (concentration reducing feeding by 50%) for the LH20 fraction was 25 $\mu g/cm^2$, comparable to a number of natural and synthetic antifeedants previously tested against this pest.[24−27] Dhilirolide L (**12**) showed the best activity of the pure compounds, giving a DC_{50} of 5.9 $\mu g/cm^2$.

When sprayed directly on second instar larvae as a 1% aqueous emulsion, the crude extract caused 47% mortality, while the previously mentioned LH20 fraction caused 63% mortality. Addition of either the crude extract or LH20 fraction to the insect's diet at 1000 ppm fwt resulted in significant sublethal effects. The crude extract reduced larval growth by 20% and adult emergence by 56%, while the dhilirolide-enriched LH20 fraction reduced larval growth by 70% and adult emergence by 87.5%.

In summary, extracts from cultures of the fungus *P. purpurogenum* have yielded 10 new meroterpenoids belonging to the new dhilirane, isodhilirane, 14,15-dinordhilirane, and 23,24-dinorisodhilirane carbon skeletons. Stable isotope feeding studies have confirmed a meroterpenoid biogenetic origin for the dhilirolides. The dhilirolides are highly functionalized and rearranged meroterpenoids with complex polycyclic frameworks. Preliminary bioassay results suggest that the dhilirolides may have considerable potential for pest management, either for direct use or as lead compounds for pesticide development.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a polarimeter with sodium light (589 nm). UV spectra were recorded with a HPLC dual λ absorbance detector. The ^{1}H and ^{13}C NMR spectra were recorded on a 600 MHz spectrometer with a 5 mm cryoprobe. ^{1}H chemical shifts are referenced to the residual DMSO-d_6 (δ 2.50), and ^{13}C chemical shifts are referenced to the DMSO-d_6 (δ 39.5) solvent peak. Reversed-phase HPLC purifications were performed using a binary HPLC pump attached to a dual λ absorbance detector or a photodiode array detector. All solvents used for HPLC were HPLC grade and were filtered through a 0.45 μm filter prior to use.

Fungal Material. The fungus *Penicillium purpurogenum* was isolated from infected fruits of *Averrhoa bilimbi* (*Averrhoa bilimbi*, L., (Oxalidaceae) as described previously.[14]

Extraction of *Penicillium perpurogenum* and Isolation of Dhilirolides A−N (1−14). As previously described, *P. purpurogenum* was cultured on potato dextrose agar (50 Petri dishes), and the resulting culture medium and attached fungal mycelium was cut into small pieces and extracted with EtOAc.[14] The EtOAc extract was concentrated in vacuo and chromatographed on Sephadex LH20 (3 cm × 95 cm) using 4:1 MeOH/CH_2Cl_2. The fractions containing the compounds of interest were combined and subjected to reversed-phase C_{18} HPLC chromatography using a CSC-Inertsil 150 Å/ODS2, 5 μm 25 × 0.94 cm column, with a linear gradient of 40−45% MeCN/H_2O over 60 min at a flow rate of 2 mL/min. Pure samples of dhilirolides A (**1**) (28.9 mg), B (**2**) (7.6 mg), C (**3**) (17.7 mg), D (**4**) (8.7 mg), F (**10**) (0.4 mg), J (**6**) (2.5 mg), L (**12**) (14.8 mg), M (**13**) (1.0 mg), and N (**14**) (0.5 mg) were obtained. Additional fractions containing impure samples of dhilirolides E (**5**), G (**7**), H (**8**), I (**9**) and K (**11**) were fractionated via isocratic C_8 reversed-phase HPLC using a Phenomenex Luna 100 Å, 5 μm 25 × 1.0 cm column. Compounds are listed with eluent systems and yields: **5** (29:71 MeCN/H_2O, 0.9 mg), **7** (9:16 MeCN/H_2O, 0.5 mg), **8** (7:13 MeCN/H_2O, 1.0 mg), **9** (9:16 MeCN/H_2O, 0.9 mg), and **11** (33:67 MeCN/H_2O, 1.4 mg).

*Dhilirolide E (**5**).* Isolated as a amorphous white powder: $[\alpha]^{20}_D$ +3.23 (*c* 0.09, MeOH); UV (MeCN) λ_{max} (ε) 206 (3103), 248 (2348) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; positive ion HRESITOFMS [M + Na]$^+$ *m*/*z* 465.1887 (calcd for $C_{25}H_{30}O_7Na$, 465.1889).

*Dhilirolide F (**6**).* Isolated as a amorphous white solid: $[\alpha]^{20}_D$ +18.1 (*c* 0.03, MeOH); UV (MeCN) λ_{max} (ε) 204 (7859), 240 (4037) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; positive ion HRESITOFMS [M + Na]$^+$ *m*/*z* 479.2050 (calcd for $C_{26}H_{32}O_7Na$, 479.2046).

*Dhilirolide G (**7**).* Isolated as a amorphous white solid: $[\alpha]^{20}_D$ +8.9 (*c* 0.05, MeOH); UV (MeCN) λ_{max} (ε) 204 (5575), 269 (4786) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; positive ion

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HRESITOFMS $[M + Na]^+$ m/z 495.1982 (calcd for $C_{26}H_{32}O_8Na$, 495.1995).

Dhilirolide H (8). Isolated as a amorphous white solid: $[\alpha]^{20}_D$ +2.92 (c 0.10, MeOH); UV (MeCN) λ_{max} (ε) 201 (1847), 240 (941) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; negative ion HRESITOFMS $[M - H]^-$ m/z 471.2014 (calcd for $C_{26}H_{31}O_8$, 471.2019).

Dhilirolide I (9). Isolated as a amorphous white solid: $[\alpha]^{20}_D$ +2.89 (c 0.09, MeOH); UV (MeCN) λ_{max} (ε) 203 (2700), 272 (2067) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; positive ion HRESITOFMS $[M + Na]^+$ m/z 453.1920 (calcd for $C_{24}H_{30}O_7Na$, 453.1889).

Dhilirolide J (10). Isolated as a clear crystalline solid: mp dec 223−225 °C; $[\alpha]^{20}_D$ +5.94 (c 0.16, MeOH); UV (MeCN) λ_{max} (ε) 206 (4349), 279 (5346) nm; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; positive ion HRESITOFMS $[M + H]^+$ m/z 441.1918 (calcd for $C_{25}H_{29}O_7$, 441.1913).

Dhilirolide K (11). Isolated as a amorphous white solid: $[\alpha]^{20}_D$ −2.26 (c 0.09, MeOH); UV (MeCN) λ_{max} (ε) 206 (883), 249 (978) nm; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; positive ion HRESITOFMS $[M + Na]^+$ m/z 479.1679 (calcd for $C_{25}H_{28}O_8Na$, 479.1682).

Dhilirolide L (12). Isolated as clear prismlike crystals: mp 271−273 °C $[\alpha]^{20}_D$ +182 (c 0.07, MeCN) UV (MeCN) λ_{max} (ε) 218 (2315) nm; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; positive ion HRESITOFMS $[M + Na]^+$ m/z 463.1745 (calcd for $C_{25}H_{28}O_7Na$, 463.1733).

Dhilirolide M (13). Isolated as a amorphous white solid: $[\alpha]^{20}_D$ +59 (c 0.02, MeOH); UV (MeCN) λ_{max} (ε) 197 (4526), 273 (132) nm; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; positive ion HRESITOFMS $[M + Na]^+$ m/z 463.1740 (calcd for $C_{25}H_{28}O_7Na$, 463.1733).

Dhilirolide N (14). Isolated as a amorphous white solid: $[\alpha]^{20}_D$ −1.4 (c 0.05, MeOH); UV (MeCN) λ_{max} (ε) 215 (2285) nm; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; positive ion HRESITOFMS $[M + Na]^+$ m/z 477.1521 (calcd for $C_{25}H_{26}O_8Na$, 477.1525).

Stable Isotope Feeding Study. For the labeling study, 1 g of [1,2-$^{13}C_2$]acetate was dissolved in 20 mL of sterile distilled H_2O and filtered through a 0.25 μm filter under sterile conditions. This solution was then added to 250 mL of autoclaved potato dextrose agar media, which was then divided evenly between 16 Petri dishes and the *P. purpurogenum* cultured as described above. The resulting culture medium was extracted with EtOAc, and dhilirolides A (**1**) (6.2 mg), B (**2**) (1.9 mg), C (**3**) (1.8 mg), D (**4**) (1.7 mg), L (**12**) (3.3 mg) and M (**13**) (2.3 mg) were isolated as described above.

Feeding Deterrent Bioassay. Freshly molted third instar larvae were starved for 4−5 h prior to each bioassay. Cabbage leaf discs of 1.5 cm diameter were painted with 10 μL of the methanolic solution of fungal extract or the compounds on each side. Control leaf discs were painted with the carrier solvent alone. One treated and one control disc (after being dried) were placed 0.7 cm apart in each compartment [4.2/3.0 cm (length/width)] of a plastic assay tray. A starved larva was introduced gently into the center of each compartment using forceps and allowed to feed. The trays were covered with plastic lids. When approximately 50% of the control leaf discs had been eaten, larvae were removed from the assay trays. Areas of control and treated leaf discs eaten were measured using Scion Image software. A feeding deterrence index (FDI) was calculated using the formula FDI = $[(C - T)/(C + T)100]$ where C and T are the control and treated leaf areas consumed by the larvae.[28]

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and NMR spectra for compounds **5**−**14** and details of the X-ray diffraction analysis of **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Sturz, A. V.; Christie, B. R.; Nowak, J. *Crit. Rev. Plant Sci.* **2000**, *19*, 1−30.

(2) Arnold, A. E.; Maynard, Z.; Gilbert, G. S.; Coley, P. D.; Kursar, T. A. *Ecol. Lett.* **2000**, *3*, 267−274.

(3) Andrews, J. H.; Harris, R. F. *Annu. Rev. Phytopathol.* **2000**, *38*, 145−180.

(4) Tucker, S. L.; Talbot, N. J. *Annu. Rev. Phytopathol.* **2001**, *39*, 385−417.

(5) Schulz, B.; Römmert, A. -K.; Dammann, U.; Aust, H. -J.; Strack, D. *Mycol. Res.* **1999**, *103*, 1275−1283.

(6) Sieber, T. N.; Sieber-Canavesi, F.; Dorworth, C. E. *Mycologia* **1990**, *82*, 569−575.

(7) Hata, K.; Futai, K.; Tsuda, M. *Can. J. Bot.* **1998**, *76*, 245−250.

(8) Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. *J. Nat. Prod.* **2004**, *67*, 257−268.

(9) Strobel, G.; Daisy, B. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 491−502.

(10) Gunatilleke, I. A. U. N.; Gunatilleke, C. V. C. *Conserv. Biol.* **1990**, *4*, 21−31.

(11) Tan, R. X.; Zou, W. X. *Nat. Prod. Rep.* **2001**, *18*, 448−459.

(12) Schulz, B.; Boyle, C.; Draeger, S.; Römmert, A.-K.; Krohn, K. *Mycol. Res.* **2002**, *106*, 996−1004.

(13) Chandra, S. *Appl. Microbiol. Biotechnol.* **2012**, *95*, 47−59.

(14) De Silva, E. D.; Williams, D. E.; Jayanetti, D. R.; Centko, R. M.; Patrick, B. O.; Wijesundera, R. L. C.; Andersen, R. J. *Org. Lett.* **2011**, *13*, 1174−1177.

(15) Flack, H. D. *Acta Crystallogr., Sect. A* **1983**, *39*, 876−881.

(16) Bernardinelli, G.; Flack, H. D. *Acta Crystallogr., Sect. A* **1985**, *41*, 500−511.

(17) Stierle, D. B.; Stierle, A. A.; Hobbs, J. D.; Stokken, J.; Clardy, J. *Org. Lett.* **2004**, *6*, 1049−1052.

(18) Iida, M.; Ooi, T.; Kito, K.; Yoshida, S.; Kanoh, K.; Shizuri, Y.; Kusumi, K. *Org. Lett.* **2008**, *10*, 845−848.

(19) Geris, R.; Simpson, T. J. *Nat. Prod. Rep.* **2009**, *26*, 1063−1094.

(20) McIntyre, C. R.; Scott, F. E.; Simpson, T. J.; Trimble, L. A.; Vederas, J. C. *Tetrahedron* **1989**, *45*, 2307−2321.

(21) Kosemura, S.; Miyata, H.; Matsunaga, K.; Yamamura, S. *Tetrahedron Lett.* **1992**, *33*, 3883−3886.

(22) Lo, H.-C.; Entwistle, R.; Guo, C.-J.; Ahuja, M.; Szewczyk, E.; Hung, J.-H.; Chiang, Y.-M.; Oakley, B. R.; Wang, C. C. C. *J. Am. Chem. Soc.* **2012**, *134*, 4709−4720.

(23) Ahmed, S. A.; Scott, F. E.; Stenzel, D. J.; Simpson, T. J.; Moore, R. N.; Trimble, L. A.; Arai, K.; Vederas, J. C. *J. Chem. Soc. Perkin Trans. 1* **1989**, 807−816.

(24) Akhtar; Isman, M. B. *J. Appl. Entomol.* **2004**, *128*, 32−38.

(25) Akhtar, Y.; Isman, M. B.; Paduraru, P. M.; Nagabadani, S.; Nair, R.; Plettner, E. *J. Agric. Food Chem.* **2007**, *55*, 10323−10330.

(26) Akhtar, Y.; Yu, Y.; Isman, M. B.; Plettner, E. *J. Agric. Food Chem.* **2010**, *58*, 4983−4891.

(27) Akhtar, Y.; Pages, E.; Stevens, A.; Bradbury, R.; da Camara, C. A. G.; Isman, M. B. *Physiol. Entomol.* **2012**, *37*, 81−91.

(28) Akhtar, Y.; Isman, M. B.; Niehaus, L. A.; Lee, C.-H.; Lee, H.-S. *Crop Prot.* **2012**, *31*, 8−14.