Novel and Insecticidal Isobutylamides from *Dinosperma erythrococca*

Zahid Latif,† Thomas G. Hartley,‡ Martin J. Rice,§ Roger D. Waigh, and Peter G. Waterman*

Phytochemistry Research Laboratories, Department of Pharmaceutical Sciences, University of Strathclyde, George St., Glasgow G1 1XW, Scotland, U.K.

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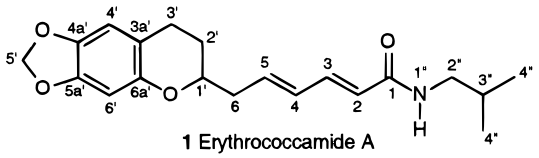
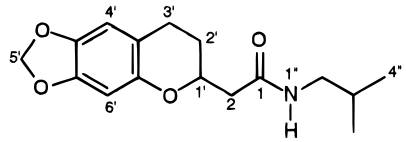
Through insecticidal bioassay-guided fractionation and isolation, five isobutylamides **1**−**5** were isolated from *n*-hexane and EtOAc extracts of the aerial parts of *Dinosperma erythrococca* (Rutaceae). The structures of compounds **1**−**5** were established through 1D and 2D NMR. Compounds **1**−**3** were identified as erythrococcamides A−C and represent two novel classes of isobutylamide. Compounds **4**−**5** were identified as *N*-(2-hydroxy-2-methylpropyl)-6-phenyl-2(*E*),4(*E*)-hexadienamide (**4**)[1,2] and *N*-(2-methylpropyl)-6-phenyl-2(*E*),4(*E*)-hexadienamide (**5**),[1,3] respectively. Compound **1** showed lethal activity against the housefly (*Musca domestica*) (kill EC_{50} = 20.2 ppm) and the tobacco budworm (*Heliothis virescens*) (kill EC_{50} = 74.2 ppm). Compound **4** showed lethal activity against *H. virescens* at 500 ppm, while compound **5** showed lethal activity against *M. domestica* at 500 ppm.

The Australian rainforest tree, formerly known as *Melicope erythrococca* F. Muell. (Rutaceae), has recently been reassigned to a new genus and renamed *Dinosperma erythrococca* (F. Muell.) T. G. Hartley.[4] Previous screening of this species had indicated that an aqueous extract of the seeds had weak insecticidal activity against the milkweed bug (*Oncopeltus fasciatus*).[5] Further examination had shown that a $CHCl_3$ extract of the aqueous extract of the aerial parts had moderate antifeedant activity against the black carpet beetle (*Attagenus piceus*); however, no followup work on these observations has since been reported.[5] Previous phytochemical studies on *D. erythrococca* (as *M. erythrococca*) reported the isolation of the common triterpene lupeol and the phenylpropanoid compound elemicin.[6]

In ongoing work to find novel insecticidal compounds from natural sources, screening of an EtOH extract of *D. erythrococca* against six economically important insect species[7] suggested it to have slight insecticidal activity. In this paper, we report the bioassay-guided fractionation of *D. erythrococca* based on insecticidal activity, which led to the isolation and identification of five isobutylamides (**1**−**5**). All five compounds were either novel or had not previously reported from natural sources.



1 Erythrococcamide A



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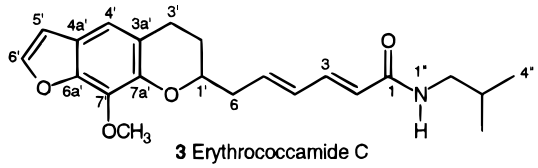
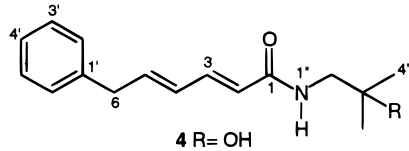


3 Erythrococcamide C



4 R= OH
5 R= H

Results and Discussion

Soxhlet extraction of the dried ground aerial parts sequentially with *n*-hexane, EtOAc, and MeOH, followed by screening of the extracts, showed the *n*-hexane and EtOAc extracts to have lethal activity against the housefly and the tobacco budworm at 10 000 ppm. Bioassay-guided fractionation of both extracts gave an active fraction from which compounds **1**−**3** were isolated. A polar fraction of the EtOAc extract also showed activity against the two-spotted spider mite. From this fraction compound **4** was isolated, although this compound was inactive against the mites when tested. Further investigation of the activity against the two-spotted spider mite was undertaken by extraction and bioassay-guided fractionation of the remaining wood of *M. erythrococca*. This led to the detection of a mite-active fraction in the *n*-hexane extract from which compound **5** was isolated.

From the ^1H and ^{13}C NMR and MS, compounds **1**−**5** could be identified as being isobutylamides (see Tables

* To whom correspondence should be addressed. Tel.: +(44)-141-548-2028. Fax: +(44)-141-552-6443. E-mail: P.G.Waterman@strath.ac.uk.

† Current Address: Xenova Discovery Ltd., 545 Ipswich Rd., Slough SL1 9DJ, U.K.

‡ Australian National Herbarium, GPO Box 1600, Canberra, ACT 2601, Australia.

§ Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire RG12 42EY, U.K.

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Table 1. ^{1}H NMR Spectral Data for Compounds **1**−**5** (in $CDCl_3$ at 400 MHz)

position	**1**	**2**	**3**	**4**	**5**
2	5.82 (d, *J* = 15.8 Hz)	2.58 (dd, *J* = 14.8, 7.9 Hz), 2.52 (dd, *J* = 14.8, 4.0 Hz)	5.81 (d, *J* = 15.0 Hz)	5.84 (d, *J* = 15.2 Hz)	5.80 (d, *J* = 15.3 Hz)
3	7.21 (dd, *J* = 15.8, 10.7 Hz)		7.24 (dd, *J* = 15.0, 10.6 Hz)	7.24 (m)	7.23 (m)
4	6.24 (dd, *J* = 15.2, 10.7 Hz)		6.30 (dd, *J* = 15.3, 10.6 Hz)	6.17 (dd, *J* = 15.2, 10 Hz)	6.15 (dd, *J* = 15.3, 9.6 Hz)
5	6.12 (dt, *J* = 15.2, 7.0 Hz)		6.19 (dt, *J* = 15.3, 7.0 Hz)	6.24 (dt, *J* = 15.1,6.2 Hz)	6.22 (dt, *J* = 15.3, 6.3 Hz)
6	2.57 (m), 2.46 (m)		2.56 (m), 2.73 (m)	3.49 (d, *J* = 6.2 Hz)	3.49 (d, *J* = 6.3 Hz)
1′	3.97 (m)	4.33 (dddd, *J* = 12.1, 7.9, 4.0, 2.2 Hz)	4.10 (m)		
2′	1.94 (m), 1.67 (m)	2.00 (m), 1.64 (m)	2.05 (m), 1.78 (m)	7.17 (br d, *J* = 7.1 Hz)	7.17 (br d, *J* = 6.9 Hz)
3′	2.72 (m), 2.64 (m)	2.65 (ddd, *J* = 16.4, 5.6, 3.2 Hz), 2.80 (ddd, *J* = 16.4, 10.2, 6.3 Hz)	2.93 (m), 2.89 (m)	7.31 (br t, *J* = 7 Hz)	7.31 (br t, *J* = 7 Hz)
4′	6.47 (s)	6.50 (s)	6.94 (s)	7.24 (m)	7.23 (m)
5′	5.84 (s)	5.86 (d, *J* = 1.4 Hz), 5.87 (d, *J* = 1.4 Hz)	6.61 (d, *J* = 2.2 Hz)		
6′	6.34 (s)	6.30 (s)	7.49 (d, *J* = 2.2 Hz)		
7′-OCH_3			4.10 (s)		
1″	5.70 (br s)	6.13 (br s)	5.48 (br t)	5.98 (br s)	5.58 (br s)
2″	3.16 (t, *J* = 6.7 Hz)	3.15 (dt, *J* = 13.4, 6.5 Hz), 3.08 (dt, *J* = 13.4, 6.5 Hz)	3.18 (t, *J* = 6.3 Hz)	3.35 (d, *J* = 6.1 Hz)	3.17 (t, *J* = 6.4 Hz)
3″	1.80 (n, *J* = 6.7 Hz)	1.80 (m)	1.80 (n, *J* = 6.4 Hz)	R= −OH, 1.71 (br s)	1.81 (n,*J* = 6.7 Hz)
4″	0.92 (d, *J* = 6.7 Hz)	0.93 (d, *J* = 6.7 Hz)	0.92 (d, *J* = 6.4 Hz)	1.24 (s)	0.93 (d,*J* = 6.7 Hz)

Table 2. ^{13}C NMR Spectral Data for Compounds **1**−**5** (in $CDCl_3$)

position	**1**	**2**	**3**	**4**	**5**
1	166.4	170.4	166.3	167.4	166.4
2	123.1	43.0	123.1	122.5	123.0
3	140.8		140.9	141.6	140.9
4	131.1		131.3	129.5	129.6
5	137.4		137.4	141.4	140.8
6	38.9		38.9	39.4	39.4
1′	75.1	73.3	75.7	139.2	139.3
2′	27.2	27.5	27.1	128.9	128.9
3′	24.8	24.7	25.2	128.8	128.8
3a′	113.3	113.4	119.6		
4′	108.3	108.5	114.2	126.6	126.6
4a′	141.5[a]	141.9[a]	122.1		
5′	100.9	101.1	106.4		
5a′	146.5[a]	146.7[a]			
6′	98.8	98.6	144.5		
6a′	149.4	148.7	146.1		
7′			133.7		
7a′			144.4		
7′-OCH_3			61.1		
2″	47.2	47.0	47.2	50.7	47.2
3″	28.8	24.7	28.8	71.3	28.8
4″	20.3	20.30, 20.29	20.3	27.5	20.3

[a] Assignments interchangeable.

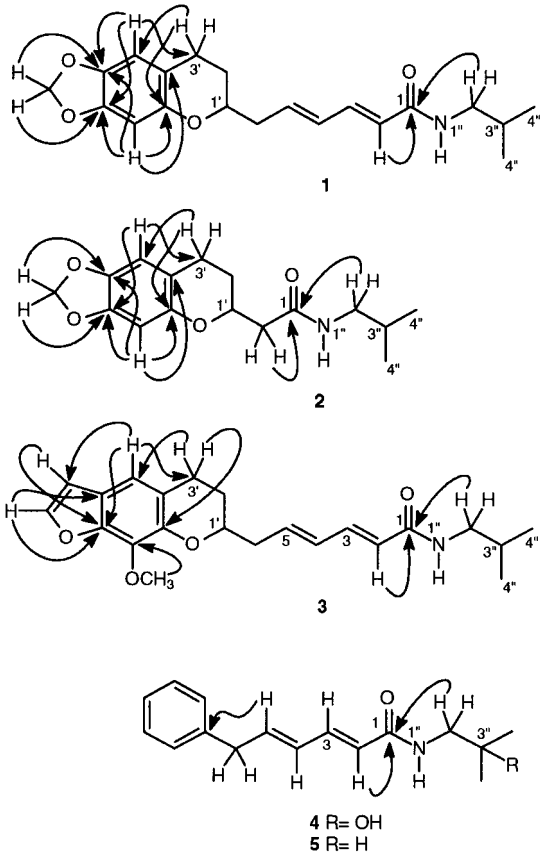


Figure 1. Important ^2J and ^3J H−C correlations seen in HMBC spectra of **1**−**5**.

1 and 2 for ^{1}H and ^{13}C NMR data, respectively). The IR spectrum of compound **1** suggested a secondary amide function (1662, 1567 cm^{-1}), while the mass spectrum gave the molecular formula $C_{20}H_{25}NO_4$ with a fragment indicative of an isobutylamide at $M^{\cdot+}$ 72 (loss of *i*-BuNH through α-cleavage at carbonyl) and the appearance of fragments at 57 amu (*i*-Bu) and 100 amu (CONH-*i*-Bu).[8]

The ^{1}H NMR and ^{1}H−^{1}H COSY spectra of **1** gave signals for an isobutylamide group (δ 5.70 (H-1″), 3.16 (H_2-2″), 1.80 (H-3″), 0.92 (2 × H_3-4″)).[9−11] In addition, the signals of a conjugated diene moiety were apparent (δ 5.82 (H-2), 7.21 (H-3), 6.24 (H-4), 6.12 (H-5)) in a trans-trans configuration as indicated by the coupling constants of 15.8 and 15.2 Hz for each pair of olefinic protons.[11] The protons of the conjugated diene moiety could be linked together from the ^{1}H−^{1}H COSY spectrum and placed adjacent to the carbonyl carbon (C-1) from a ^2J HMBC correlation (Figure 1) from the olefinic proton H-2 to C-1. The last proton of the conjugated diene chain (H-5) showed coupling to a methylene group H_2-6 (δ 2.57, 2.46), which, in turn, coupled to a deshielded, oxygen-bearing methine H-1′ (δ 3.97). H-1′ exhibited further coupling to methylene protons H_2-2′ (δ 1.94, 1.67), which, in turn, showed coupling to methylene protons of H_2-3′ (δ 2.72, 2.64). From H_2-3′ no further

coupling was seen in the $^1H-^1H$ COSY spectrum, indicating that the carbon adjacent to H_2-3′ was quaternary. The ^{13}C resonances for the partial structures were assigned from the HC-COBI experiment.

Analysis of the aromatic region of the ^{13}C NMR spectrum showed the presence of six aromatic carbons. Four quaternary aromatic carbons were seen, C-3a′ (δ 113.3), C-4a′ (δ 141.5), C-5a′ (δ 146.5), and C-6a′ (δ 149.4), the latter three being deshielded due to bonding to oxygen atoms. Two aromatic methines were observed at δ 108.3 (C-4′) and 98.8 (C-6′). The lack of visible coupling between the two aromatic protons H-4′ (δ 6.47) and H-6′ (δ 6.34) and the presence of only one aromatic ring in the molecule suggested that the aromatic protons were para to one another. A methylenedioxy group (H_2-5′ δ 5.84, δ_C 100.9) could be identified from the 1D NMR spectra, while the HMBC experiment (Figure 1) revealed these protons to show ^3J correlations to the aromatic carbons C-4a′ (δ 141.5) and C-5a′ (δ 146.5).

The structure of compound **1** was finally determined from the HMBC experiment (Figure 1), which allowed the partial structures to be linked together. The attachment of the methylene C-3′ to the quaternary C-3a′ was shown through ^3J correlations of H-3′ to C-4′ and H-4′ to C-3′, thereby establishing C-3a′ as the non-oxygen-bearing quaternary carbon (δ 113.3). Two of the oxygen-bearing quaternary carbons were accounted for as part of the methylenedioxy ring. The remaining oxygen must be attached to C-6a′ (δ 149.4), bridging the gap between C-6a′ and the deshielded oxygen-bearing methine H-1′/C-1′ (δ_H 3.97, δ_C 75.1) to form a benzopyran moiety and give the final structure **1**. A NOESY experiment was carried out in an attempt to determine whether H-1′ was axial or equatorial, but no firm conclusions could be made from the NOE correlations seen.

The ^1H and ^{13}C JMOD spectra of compound **2** were very similar to those seen for **1**, showing the presence of the (methylenedioxy)benzopyran moiety and the isobutylamide function but lacking the conjugated diene. The $^1H-^1H$ COSY and HMBC experiments allowed the various partial structures to be linked together. The $^1H-^1H$ COSY spectrum showed a correlation from the methylene group H_2-2 (δ 2.58, 2.52) to the sp^3 oxymethine H-1′ (δ 4.33) of the pyran ring and from H-1′ to the adjacent methylene protons H_2-2′ (δ 1.64, 2.00) and then H_2-3′ in the pyran ring (δ 2.65, 2.80). From the HMBC experiment, the correlations shown by the H_2-2 protons confirmed that this methylene joined the pyran ring and the isobutylamide function, through ^2J correlations between H-2 and C-1 (δ 170.4) and C-1′ (δ 73.3) and a ^3J correlation to C-2′ (δ 27.5). Finally, the H-2″ protons showed a ^3J correlation to C-1 (δ 170.4), confirming the attachment of the isobutylamine function to the carbonyl group.

The ^1H and ^{13}C NMR spectra revealed compound **3** to also be similar to compound **1**. Similarities included a benzopyran moiety and the 2(*E*),4(*E*)-hexadieneisobutylamide group, with novel features being the presence of an aromatic methoxyl group and a furan moiety. In the ^{13}C NMR spectrum the methoxyl group resonated at δ 61.1, which indicated that the ortho positions were both substituted.[12] This suggested that the furan ring was in a linear configuration with respect to the benzopyran moiety. The HMBC experiment (Figure 1) allowed the piecing together of the molecule and final determination of the structure. The significant correlations came from the sole aromatic proton H-4′, which showed ^3J correlations with the methylene C-3′ (δ 25.2) and with the oxygen-bearing aromatic carbons at C-6a′ (δ 146.1) and C-7a′ (δ 144.4), the latter being part of the pyran ring. In addition, a ^3J correlation was seen between the aromatic proton H-4′ and the furan methine H-5′ at δ 106.4, which meant that the oxygen substituent of the furan ring was meta to the aromatic proton, which was further substantiated by the fact that the furan proton H-5′ was relatively shielded at δ 6.61. Had the methoxyl group been in the C-4′ position, then the H-5′ proton would have been deshielded to ca. δ 7 due to the peri-deshielding effect of the methoxyl. This is analogous to the ^1H NMR chemical shifts seen with the furanocoumarins bergapten (**6**) and xanthotoxin (**7**).[13]

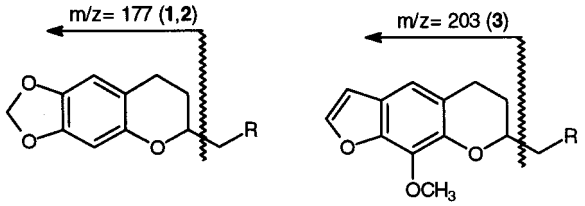
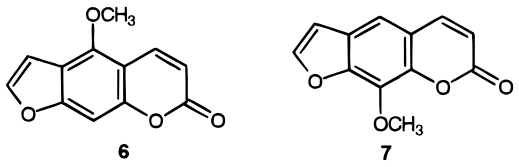
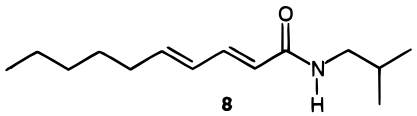


Figure 2. Probable major fragments seen from the mass spectra of compounds **1**−**3**.



6 **7**



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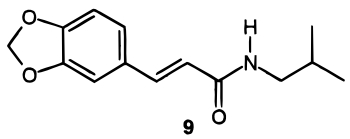

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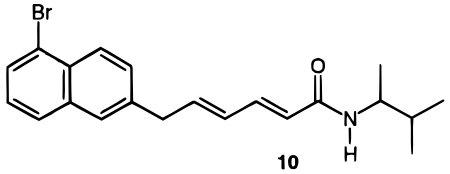

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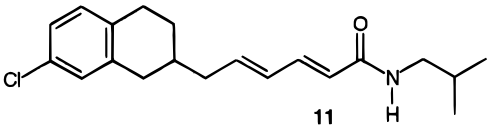

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The mass spectra of compounds **1**−**3** gave typical fragmentation patterns for isobutylamides as described above but also showed major fragments that could be ascribed to cleavage of the benzopyran moiety from the amide side chain (Figure 2).

Both the ^1H and ^{13}C NMR spectra showed compounds **4** and **5** to have the conjugated 2(*E*),4(*E*)-hexadiene

Table 3. Insecticidal Activity of Compounds **1**−**5** and **8**−**9**

compd	insecticidal activity[a]	other observations
1	housefly kill EC_{50} = 20.2 ppm. rel potency = 3% of cypermethrin tobacco budworm kill EC_{50} = 74.2 ppm. Rel potency = 9.9% of profenofos	also showed housefly knockdown activity at 500 ppm
2	inactive at 500 ppm	
3	inactive at 500 ppm	
4	gave 80−100% kill against tobacco budworm at 500 ppm[b]	
5	housefly kill EC_{50} = 59.9 ppm.[c] Rel potency = 1.8% of cypermethrin[b]	also showed housefly knockdown activity at 500 ppm
8	inactive at 1000 ppm	also showed housefly knockdown activity at 2500 ppm
9	inactive at 2500 ppm	

[a] Tested against the six pest species listed in ref 7. [b] Due to shortage in compound, only preliminary activity-studies done. [c] Results are for the synthetic compound made during other research carried out by Zeneca Agrochemicals Ltd.[31]

system of **1** together with a monosubstituted aromatic ring. In **4**, an isobutylamide group was not immediately apparent. Two deshielded 2H doublets were observed (δ 3.35, 3.49) together with a deshielded 6H singlet (δ 1.24), the latter suggestive of two methyl groups attached to a quaternary oxygen-bearing carbon. In the $^1H-^1H$ COSY experiment, starting from H-2 (olefinic adjacent to carbonyl), it was possible to trace the conjugated diene function to H-5, which showed a correlation to a 2H doublet at δ 3.49 (H_2-6). The N−H proton meanwhile showed a correlation to the 2H doublet at δ 3.35 (H_2-2″). The JMOD ^{13}C NMR revealed the presence of two nonaromatic quaternary carbons, one of which could be identified as the carbonyl C-1 (δ 167.4) while the other (δ 71.3) must be the carbon to which the two methyl groups were attached and which is deshielded due to an OH substituent. The 2D HC-COBI allowed the assignment of direct ^1J C−H correlations, and HMBC (Figure 1) facilitated the complete assignment of the structure. The correlations of importance from the HMBC experiment showed attachment of the aromatic ring to the 2(*E*),4(*E*)-hexadiene chain as demonstrated by a ^3J correlation between H-5 and the aromatic quaternary C-1′ (δ 139.3). Similarly, the protons H_2-6 gave a ^3J correlation with the carbon C-2′. The protons H_2-2″ showed correlations to C-1, C-3″, and C-4″. Compound **4** was thus identified as *N*-(2-hydroxy-2-methylpropyl)-6-phenyl-2(*E*),4(*E*)-hexadienamide. Comparison of the physical and spectroscopic properties of compound **4** with those quoted in the literature[1,2] for the synthetic compound showed them to be identical.

Analysis of the ^1H and ^{13}C NMR spectra of compound **5** showed the same unsubstituted isobutylamide group as **1**−**3**, together with the phenyl-2(*E*),4(*E*)-hexadienamide unit of **4**. From 1D and 2D NMR experiments compound **5** was confirmed as *N*-(2-methylpropyl)-6-phenyl-2(*E*),4(*E*)-hexadienamide and showed physical and spectral properties identical to the synthetic compound reported.[1−3]

The insecticidal activities of compounds **1**−**5** are shown in Table 3 together with those of the isobutylamides pellitorine (**8**) isolated from *Zanthoxylum tessmannii*[14] and fagaramide (**9**) isolated from *Dinosperma melanophloia* (formerly *Melicope melanophloia*).[15] Various naturally occurring isobutylamides have been shown to be active against insect species.[16,17,20] Synthetic work on isobutylamides used compound **5** as a lead compound and structural variations led to improved activity, the most potent compounds being **10** and **11**.[3,18,19]

The results found in this work mirror the synthetic studies, with **1** being implicated as the major active compound of the crude extract. The active compounds all possessed conjugated 2(*E*),4(*E*)-hexadienamide moieties, which are crucial to activity.[3,18−20] The absence of the diene function in **2** and **9** resulted in a complete loss in activity. Compound **1** was shown to be more potent than **5** and to possess a broader range of activity. This suggested that substitution of the aromatic ring can lead to further increases in potency, a view substantiated by the synthetic work.[3,18,19] Compound **3** was inactive at 500 ppm on all screens, which suggested a limit to substitution on the aromatic ring above which total loss of activity is seen, possibly due to restricted access to the receptor site.

The close resemblance of the aromatic moiety of **3** to xanthotoxin (**7**) may possibly allow the plant to exploit two modes of insecticidal action. Furanocoumarins such as bergapten (**6**) and xanthotoxin (**7**) have been reported to have antifeedant and insecticidal activity,[21−24] and the presence of hexadienamide and furanocoumarin-like moieties in **3** could lead to a dual mode of action against pest species that *D. erythrococca* encounters in the wild. The substitution of **1** with a methylenedioxy moiety may enhance the activity of this compound through inhibition of metabolism, an activity characterized by the sesamin-type insecticide synergists.[25,26]

Compounds **4** and **5** came from mite active fractions but when tested did not show any activity against mites. These fractions were then recombined and tested, but activity against the two-spotted spider mite could not be reestablished. The isolation of two structurally very similar isobutylamides from fractions that showed activity against mites suggests that they may be implicated in the activity seen for the fractions. The possibility of synergism between the isobutylamides and compound(s) unknown (that are possibly unstable) cannot be entirely ruled out, although the lack of activity in the recombined fractions points to another compound being responsible for activity in the mite screen.

In an assay measuring relative potency, compound **1** possessed 3% of the activity of the pyrethroid insecticide cypermethrin against the housefly. It has been reported that **11** gave 40% of the activity of the pyrethroid permethrin against houseflies.[19] A general comparison of activity between permethrin and cypermethrin suggested that cypermethrin was approximately three to four times more potent than permethrin,[27] which in turn suggests that **1** has approximately 9−12% of the activity of permethrin.

The taxonomic placements of the genera *Euodia* and *Melicope* in the Rutaceae are currently under review.[28] Some northern *Euodia* species have been transferred to the closely related "proto-rutaceous" genus *Tetradium*.[29] For the family Rutaceae as a whole, the distribution of isobutylamides has been restricted to the two proto-rutaceous genera *Zanthoxylum* and *Tetradium*[30] and to the species *Dinosperma melanophloia* (formerly *Melicope melanophloia*),[15] which is to be placed into the newly reassigned genus *Dinosperma* along with *D. erythrococca* on morphological grounds.[4,28] This work represents the second report of the isolation of isobutylamides from this newly created genus. The compounds isolated in this study mark an advance in biosynthetic complexity for this class of compound as a whole, employing both cinnamate and acetate pathways (probably with mevalonate involvement in the formation of the furan ring) to yield more potent insecticidal compounds. The isobutylamides may also prove to be chemotaxonomic markers to identify other members of this genus.

Experimental Section

General Procedures. Melting points (uncorrected) were determined on a Kofler hot-stage apparatus. UV spectra were recorded using a Perkin-Elmer 552 spectrophotometer with the sample dissolved in MeOH. IR spectra were recorded as KBr disks on a Matson Genesis series FT-IR spectrophotometer. Optical rotations were measured on a Bellingham and Stanley ADP220 instrument and the samples dissolved in $CHCl_3$. HREIMS were run on a JEOL JMS-AX505HA double-focusing instrument at 70 Ev. EIMS were run on a JEOL DX303 in EI mode. NMR spectra (both one- and two-dimensional) were obtained on a Bruker AMX 400 (400 MHz for ^1H) spectrometer, all samples dissolved in $CDCl_3$. Vacuum−liquid chromatography (VLC) was carried out using Merck silica gel 60H. Column chromatography was carried out using silica gel (Merck 0.063−0.2 μm) or Sephadex LH-20 (Pharmacia). Preparative HPLC was performed using a Gilson 601 system with a Spherisorb ODS2 C-18 column (25 mm i.d. × 250 mm). Analytical and preparative TLC (PTLC) were performed on Merck silica gel 60 F_{254} (0.2 mm thick) and Merck silica gel 60 PF_{254} (1 mm thick) plates, respectively, and the plates visualized under UV (254 and 366 nm) and by spraying with 1% vanillin−H_2SO_4 and then heating.

Plant Material. Aerial parts of *D. erythrococca* (F. Muell.) T. G. Hartley were collected from rainforest (at 500 m altitude), Atherton Tableland, Cook District, Queensland. A voucher specimen (voucher no. Waterman 91/3) was deposited at the Australian National Herbarium, Canberra.

Testing for Insecticidal Activity. The initial screening involved counting out approximately 10 of the individual test species[7] into a container prepacked with an adequate food supply. The extract/fraction/compound was dissolved in a 50:50 mixture of acetone and an aqueous suspending agent, and the complete mixture was sprayed into the container holding the test species. After a period of time (different for each species, typically 3−5 days), the assay was assessed for percentage lethality and scored. The control experiment involved spraying a similar container holding insects and food supply with the same volume of carrier and assessing mortality after the same period of time. Crude extracts were screened at a concentration of 10 000 ppm with fractions being tested at 1000−5000 ppm. Pure active compounds in sufficient quantity were tested further at varying concentrations to ascertain EC_{50} values.

Extraction. The dried, ground plant material was extracted using a Soxhlet apparatus. The initial EtOH extract showed weak activity against the housefly and the tobacco budworm. Large-scale extraction was done sequentially with *n*-hexane followed by EtOAc and then MeOH. Two different extracts were prepared, one for all aerial parts, the second for the wood.

Bioassay-Guided Fractionation and Isolation. Results from the insecticidal bioassays showed the *n*-hexane and EtOAc extracts to possess lethal activity against the housefly and the tobacco budworm. The EtOAc extract also showed lethal activity against the two-spotted spider mite. VLC fractionation of the *n*-hexane extract on silica gel was carried out, eluting with *n*-hexane and then *n*-hexane with increasing amounts of EtOAc. Fractions were analyzed by TLC and similar fractions combined. The fractions were tested for biological activity, and the fraction F3 (eluted with 30%-70% EtOAc) was active against the housefly and the tobacco budworm. This fraction was then subjected to column chromatography using Sephadex LH-20, eluting with $CHCl_3$ and collecting 40 mL fractions. Fraction 2 again showed lethality against the housefly and the tobacco budworm. Fraction 2 was dissolved in 1:1 *n*-hexane/EtOAc, and a precipitate formed that was filtered off to give compound **1** (18 mg). The solution was subjected to PTLC (*n*-hexane/EtOAc 4:1) to give compounds **2** (8 mg) and **3** (4 mg).

The EtOAc extract showed lethal activity against the two-spotted spider mite, the housefly, and the tobacco budworm. VLC fractionation of the *n*-hexane extract on silica gel was carried out, eluting with *n*-hexane and then *n*-hexane containing increasing amounts of EtOAc, and finally with MeOH. The activity against the housefly and tobacco budworm could be attributed to the presence of **1**. The activity against the two-spotted spider mite was found to occur in a more polar fraction, eluted from the VLC column using 60% EtOAc in MeOH to 100% MeOH. The active fraction was twice subjected to column chromatography using Sephadex LH-20, eluting with $CHCl_3$ with increasing amounts of MeOH and collecting 40 mL fractions. The fractions were analyzed by TLC and like fractions combined and screened for activity. Compound **4** was isolated from the active fraction by preparative HPLC using a Gilson 601 system with a Spherisorb ODS2 C-18 column (25 mm i.d × 250 mm) and eluting with 40% MeOH in H_2O, collecting the major peak at 10.3 min. Compound **4** was inactive against mites when screened at a concentration of 2500 ppm. A reexamination of the mite activity was undertaken by extracting the remaining wood of *D. erythrococca* as above. The activity against mites was concentrated in the *n*-hexane extract. VLC of the *n*-hexane extract led to an active fraction eluted by 20−70% EtOAc in *n*-hexane. The active fraction was subjected to gel chromatography using Sephadex LH-20 eluting with $CHCl_3$ and collecting 40 mL fractions.

Fraction 2 was found to be active, and this was subjected to column chromatography using silica gel (eluting *n*-hexane/EtOAc). The mite-active fraction was eluted using 22−30% EtOAc in *n*-hexane. The major compound from this fraction was compound **5**, which precipitated out of a $CHCl_3$/MeOH mixture.

Erythrococcamide A (1) [6-[3,4-dihydro-6,7-(methylenedioxy)-2*H*-1-benzopyran-2-yl]-*N*-(2-methylpropyl)-2(*E*),4(*E*)-hexadienamide]: needles from hexane/EtOAc; mp 160−161 °C; $[\alpha]_D$ (*c* 0.01, $CHCl_3$) +133°; UV λ_{max} (MeOH) 257, 310 nm (sh); IR ν_{max} (KBr disk) 3391, 3283, 2959, 1662, 1635, 1621, 1567, 1513, 1486 cm^{-1}; EIMS *m*/*z* 343 [M^+] (62), 271 (6), 193 (23), 177 (100), 167 (55), 151 (42), 141 (11), 135 (24), 119 (18), 100 (12), 91 (17), 77 (12), 68 (23), 57 (58), 28 (47).

Erythrococcamide B (2) [2-[3,4-dihydro-6,7-(methylenedioxy)-2*H*-1-benzopyran-2-yl)-*N*-(2-methylpropyl]acetamide]: pale yellow solid; mp 95 °C; $[\alpha]_D$ (*c* 0.001, $CHCl_3$) +73°; UV λ_{max} (MeOH) 256 nm; IR ν_{max} (KBr disk) 3432, 2958, 1643, 1508, 1487, 1477 cm^{-1}; HREIMS 291.1468 (calcd for $C_{16}H_{21}NO_4$, 291.1471); EIMS *m*/*z* 291 [M^+] (100), 220 (18), 218 (25), 202 (10), 190 (14), 177 (32), 176 (23), 175 (24), 162 (36), 151 (56), 149 (42), 141 (20), 134 (23), 123 (28), 121 (30), 68 (59), 57 (70).

Erythrococcamide C (3)[6-(5,6-dihydro-9-methoxy-7*H*-furo[3,2-*g*]-1-benzopyran-7-yl)-*N*-(2-methylpropyl)-2(*E*),4(*E*))-hexadienamide]: pale yellow wax; mp 42 °C; $[\alpha]_D$ (*c* 0.001, $CHCl_3$) +185°; UV λ_{max} (MeOH) 255, 260, 270, 295 nm; IR ν_{max} (KBr disk) 3440, 3324, 2958, 1631, 1624, 1573, 1504,1496, 1478, 1296, 1153 cm^{-1}; HREIMS 369.1969 (calcd for $C_{22}H_{27}NO_4$, 369.1940); EIMS *m*/*z* 369 [M^+] (73), 345 (11), 343 (14), 291 (54), 267 (18), 256 (14), 218 (18), 203 (100), 177 (53), 176 (20), 167 (79), 151 (55), 57 (91).

***N*-(2-Hydroxy-2-methylpropyl)-6-phenyl-2(*E*),4(*E*)-hexadienamide (4):** yellow waxy solid; mp 77 °C; UV λ_{max} (MeOH) 257 nm; IR ν_{max} (KBr disk) 3440, 2876, 1658, 1627, 1550, 1460, 1377, 1257 cm^{-1}; EIMS *m*/*z* 259 [M^+] (13), 201 (28), 171 (12), 143 (23), 128 (23), 115 (10), 110 (100), 91 (18), 59 (14).

***N*-(2-Methylpropyl)-6-phenyl-2(*E*),4(*E*)-hexadienamide) (5):** needles; mp 116 °C; UV λ_{max} (MeOH) 256 nm; IR ν_{max} (KBr disk) 3305, 2960, 2875, 1650, 1608, 1540, 1475 cm^{-1}; HREIMS 243.1566 ($C_{16}H_{21}NO$ requires 243.1623); EIMS *m*/*z* 243 [M^+] (75), 171 (54), 152 (32), 143 (28), 128 (91), 84 (100).

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(7) The insect test species and their ages are as follows: two-spotted spider mite (*Tetranychus urticae*), adult; peach/potato aphid (*Myzus persicae*), mixed instars; housefly (*Musca domestica*), adult; tobacco budworm (*Heliothis virescens*), larvae; lesser armyworm (*Spodoptera exigua*), larvae; root knot nematode (*Meloidogyne incognita*), juveniles.
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