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Oncidinol—a novel diacylglycerol from *Ornithophora radicans* Barb. Rodr. (Orchidaceae) floral oil

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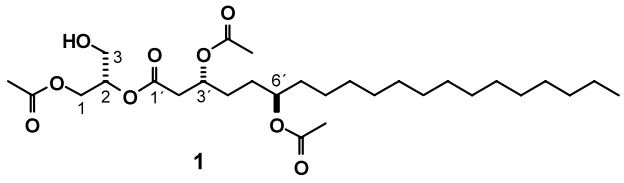
Abstract—The structure and absolute configuration of a novel diacylglycerol, (2*S*,3′*R*,6′*R*)-1-acetyl-2-[3′,6′-diacetoxyeicosanyl)-glycerol **1**, named oncidinol, from the *Ornithophora radicans* floral oil was elucidated from spectroscopic data and by applying the Mosher method.

Without flower pollination, many plants would not be able to reproduce, and many insects would not be able to obtain enough protein or carbohydrate to survive. This successful mutualistic interaction, which began with the rise of the angiosperm in the Mesozoic era,[1] relies on pollen vectors or pollinators (animals) searching for food (nectar, lipids, pollen) and other means to survive (e.g. resin for nest building). Pollinators are attracted by physical (color) and chemical signals (fragrant compounds). These signals lure insects to the flowers, where they unintentionally pollinate them. To fully understand the pollination process one has to go beyond the observations and probe the chemical signals that are at the basis of the communication between plants and the pollen vectors. Most investigations in this area are not complete from the chemical point of view and we believe that chemistry can explain most of these relationships although it is not easy to unveil the invisible pathways of these chemical communications.



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Nectar and pollen are common floral rewards while floral oils are rare and only available in a few plant families. The floral oils are secreted by a structure called the elaiophor, which in orchid flowers is located in a small region called the callus, located at the base of the floral lip. These floral oils are collected by special bees that use the floral oils for nest construction and food. Flower pollination occurs during floral oil collection[2] and we were thus motivated to study the chemical composition of floral oils belonging to 30 different species of the subtribe Oncidiinae. We have found that the major components of the floral oils of *Oncidium pubes* Lindl.,[3] *O. hookeri* Rolfe, *O. cornigerum* Lindl., *O. truncatum* Pabst, *O. amictum* Lindl., *O. longicornu* Mutel, *O. welteri* Pabst, *Baptistonia echinata* Barb. Rodr. and *Ornithophora radicans* Barb. Rodr. are asymmetrically substituted diacylglycerols.[4] Asymmetrically substituted diacylglycerols are known to activate the protein kinase C (PKC), responsible for the cellular signal transductions that promote lipid hydrolysis and phosphorylation of a variety of target proteins which control growth and cellular differentiation.[5] Therefore the role of these floral oils in the bees' lives may be more important than just nest material. Motivated by these considerations we proposed to fully characterize some of these diacylglycerols, which have previously not been characterized and investigate their importance in the bees life-cycle for future investigation. We now report the structure and absolute configuration of oncidinol **1**, a novel diacylglycerol, (2*S*,3′*R*,6′*R*)-1-acetyl-2-[3′,6′-diacetoxyeicosanyl)-glycerol, a major constituent from the *Ornithophora radicans* (Orchidaceae) floral oil.

Keywords: Orchidaceae; *Ornithophora radicans*; floral oil; diacylglycerol; floral rewards.
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Flowers of *Ornithophora radicans* were obtained from the botanical orchid nursery of the Escola Superior de Agricultura Luiz de Queiroz, ESALQ-USP, (collection number 27.656/10-4-75) in Piracicaba-SP, Brazil, in February 2003. The floral oil secreted from the callus was collected by applying different techniques: using glass capillaries and by extracting the flowers with ethyl acetate. Analysis of the components present in the mixtures revealed that they were analogous. We therefore chose the solvent extraction technique which was less time consuming. Thus, the ethyl acetate extract (1.8 g) was obtained from 21.3 g of freshly collected flowers. Purification of the crude extract by silica column chromatography eluting with hexane/ethyl acetate/triethylamine (1:1:0.01 v:v), to prevent 1,2- to 1,3-diacylglycerol isomerization,[6] furnished a 90% pure diacylglycerol (210 mg), the major mixture component, which was characterized by ^{1}H and ^{13}C NMR (Table 1). Further purification was unsuccessful producing the rearranged diacylglycerol **2** as confirmed by ^{1}H NMR analysis.[7] The molecular ion at m/z 545 [M+H] was observed by ESMS, which, together with other spectroscopic data, is consistent with the molecular formula $C_{29}H_{52}O_9$. 1D (^{1}H and ^{13}C) and 2D NMR (H,H COSY, H,C HSQC, H.C HMBC) spectroscopic analyses were of major importance in determining the structure of the diacylglycerol **1** (Table 1) in a step-by-step approach with the assignment of the glycerol moiety substitution pattern being the first problem solved. The ^{1}H NMR spectrum (Table 1) contained signals at δ 5.08 (m, 1H, H-2), δ 4.25 (dd, 2H, J=12.1, 5.9 Hz, H-1) and δ 3.75 (bs, 2H, H-3) that are characteristic of 1,2-diacylglycerol derivatives.[8] Among the four carbonyl signals observed in the ^{13}C NMR spectrum, three (δ 170.5, δ 170.6 and δ 170.8) showed long range correlations to methyl groups resonating at δ 2.04, δ 2.05 and δ 2.07, and were therefore assigned to acetoxy groups. The ^3J heteronuclear correlation between the hydrogens at δ 4.25 (H-1, dd, ^3J) and one of the carbonyls was the major evidence of an acetoxy substituent at position C-1. Evidence for the attachment of the long acyl chain through C-2 were obtained from the long distance heteronuclear correlations between the carbonyl at δ 169.7 and the hydrogen at δ 2.60 (H-2′,m, ^2J) and hydrogen at δ 5.08 (H-2, m, ^3J). With the substitution of the glycerol moiety well established we focused our attention on the spectroscopic evidence supporting the substitution pattern of the fatty acid moiety. Based on the ^{1}H NMR analysis the more deshielded methine signal was assigned to H-3′ (δ 5.20), based on its two homonuclear correlations: (a) to the hydrogen signal at δ 2.60 (two hydrogens on carbon-2′) characteristic of β-acetoxy substituted long chain fatty acids[3] and (b) to the two hydrogens on C-4′ resonating at δ 1.63. Following the homonuclear hydrogen connectivity sequency (COSY) we observed that H-6′ at δ 4.84 was connected to H-5′ resonating at δ 1.52 and that H-5′ showed a correlation signal with H-4′ at δ 1.63, thus composing a spin system from H-2′ to H-6′, establishing a two carbon distance between the two acetoxy groups along the fatty acid residue. NMR heteronuclear C,H correlations the ^2J between H-5′ (δ 1.52) and C-6′ (δ 73.8), the ^2J between H-4′ (δ 1.63) and C-3′ (δ 70.2) and the ^2J between H-2′ (δ 2.60) and the carbonyl at δ 169.7 (C-1′), which confirmed the 3′,6′-diacetoxy substitution pattern of the fatty acid moiety.

Notwithstanding the satisfactory spectral characterization of compound **1**, further investigation was required to determine the absolute configuration of the three asymmetric centers, C-2, C-3′ and C-6′. It was also important to determine the optical rotation or circular dichroism curve using a pure sample.

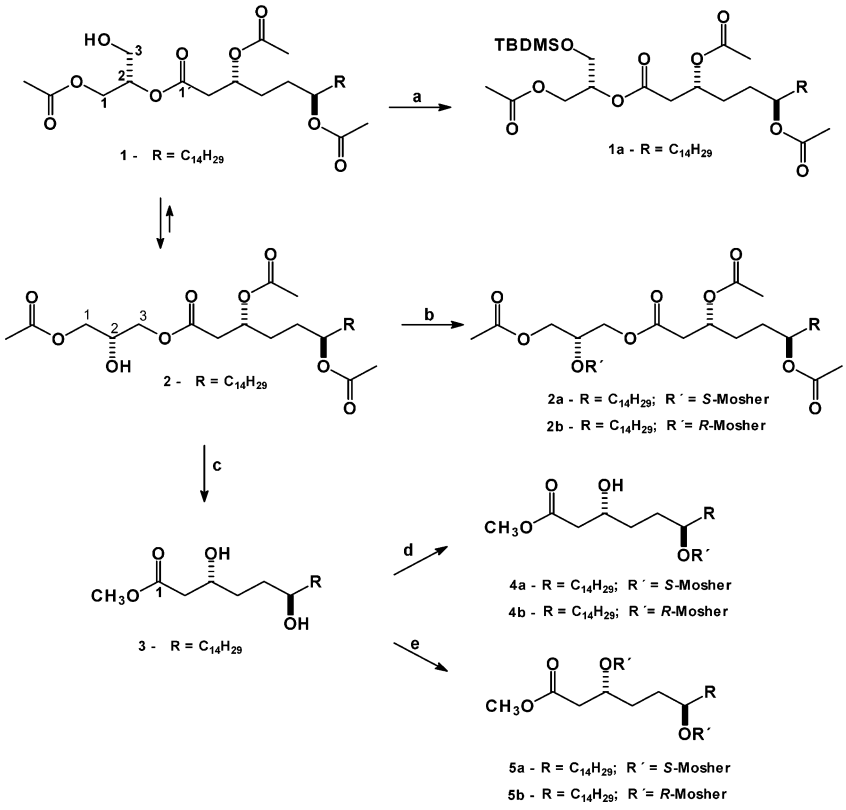
To prevent the isomerization which had occurred during all purification attempts, we derivatized the diacylglycerol **1** as its ether *t*-butyldimethylsilyl **1a**,[9] which was then easily separated from 1,3-diacylglycerol **2** by column chromatography. Pure **1a** displayed a positive Cotton effect at 225.6 nm in the CD spectrum and a specific optical rotation of $[\alpha]_D^{20}$ +16.5 (c=2, (g/mL), hexane).

To determine the absolute configuration of the three asymmetric centers we applied Mosher's methodology[10] by strategically modifying one chiral center at a time, as depicted in Scheme 1, based on the known rearrange-

Table 1. ^{1}H (500 MHz, $CDCl_3$)[a], ^{13}C NMR (125 MHz, $CDCl_3$)[b] and HMBC data for oncidinol 1

Carbon	δ_C	δ_H	HMBC (^{1}H→^{13}C)	Carbon	δ_C	δ_H	HMBC (^{1}H→^{13}C)
1′	169.7			19′	22.6	1.25 (m)	
2′	39.5	2.60 (m, 2H)	C1′	20′	14.1	0.87 (t, 3H, J=6,6)	
3′	70.2	5.20 (m, 1H)		1	62.1	4.25 (dd, 2H, J=12.1, 5.9)	$\underline{C}OCH_3$
4′	33.9	1.63 (m, 2H)	C3′	2	72.7	5.08 (m, 1H)	C1′
5′	33.7	1.52 (m, 2H)	C6′	3	61.2	3.75 (m, 2H)	
6′	73.8	4.84 (m, 1H)		$\underline{CH}_3CO_2$-C1	20.7	2.07 (s, 3H)	
7′	34.1	1.52 (m)		$CH_3\underline{C}O_2$-C1	170.6		
8′	25.3	1.25 (m)		$\underline{CH}_3CO_2$-C3′	21.1	2.04 (s, 3H)	
9′	29.5	1.25 (m)		$CH_3\underline{C}O_2$-C3′	170.8		
10′–16′	29.6	1.25 (m)		$\underline{CH}_3CO_2$-C6′	21.2	2.05 (s, 3H)	
17′	29.5	1.25 (m)		$CH_3\underline{C}O_2$-C6′	170.5		
18′	31.9	1.25 (m)					

[a] Chemical shifts are referenced to TMS (δ 0.00).
[b] Chemical shifts are referenced to $CDCl_3$ (δ 77.0).



Scheme 1. Preparation derivatives of **1**. *Reagents and conditions*: (a) TBDMSiCl/DMAP/Et_3N/CH_2Cl_2 2 h at rt (7%); (b) DCC/DMAP/CH_2Cl_2/α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA), 5 min at 0°C and 5 h at reflux, (72%); (c) LiOH·H_2O/THF/MeOH/H_2O 30 min at 0°C and 24 h at rt, CH_3COOH, 70% v/v, pH = 7, diazomethane, 0°C (36%); (d) DCC/DMAP/CH_2Cl_2/MTPA, 15 min at 0°C and 15 h at rt, (14%); (e) DCC/DMAP/CH_2Cl_2/MTPA, 5 min at 0°C and 24 h at reflux (22%).

ment mechanism of 1,2- into 1,3-diacylglycerol derivatives, which occurs with complete retention of configuration.[6] Esterifications of the 1,3-diacylglycerol derivative **2** with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) in the presence of 4-dimethylaminopyridine and 1,3-dicyclohexylcarbodiimide produced **2a** and **2b**.[11] From the chemical shift differences $\Delta\delta^{SR}$ (Table 2) we concluded that the configuration is 2-*R* in **2** and consequently is 2-*S* in the natural compound **1**.

Table 2. Partial ^{1}H NMR (500 MHz, $CDCl_3$) data of the (*S*)- and (*R*)-Mosher ester derivatives of compound **2**[a,b]

Hydrogen	δ_H		$\Delta\delta_{S-R}$
	2a	**2b**	
***CH*$_3$CO$_2$-C1**	2.07	1.98	+0.09
1	4.42	4.32	+0.10
	4.19	4.11	+0.08
3	4.34	4.41	−0.07
	4.15	4.20	−0.05
2′	2.49	2.54	−0.06

[a] Assignments were aided by 2D NMR (COSY, HSQC and HMBC experiments).

[b] Chemical shifts are referenced to TMS (δ 0.00).

Assessing the absolute configurations of carbons -3′ and -6′, of **1** required careful derivatization because the absolute configuration determination of polyols by applying Mosher's methodology is not straightfoward and the anisotropic effects of each MTPA residue have to be evaluated separately. This can be assessed by sequential derivatization and keeping in mind that the observed $\Delta\delta^{SR}$ for certain hydrogens is the sum of the anisotropic effects of all MTPAs present, leading to a result that is different from the $\Delta\delta^{SR}$ obtained for the polyols with only one MTPA.[12]

Taking into consideration the above strategy we first hydrolyzed compound **2** in THF/MeOH/H_2O (3:1:1) in the presence of LiOH[13] furnishing 3,6-dihydroxyeicosanoic acid, which was methylated with diazomethane, producing **3** in 36% yield[14] (Scheme 1).

The selective esterification of the 6-OH of **3** with (*S*)-MPTA and (*R*)-MPTA produced derivatives **4a** and **4b**,[15] which were characterized by the H-6 signals at δ 5.09. The chemical shifts and the difference $\Delta\delta^{SR}$ are shown in Table 3. From these results we suggest a 6-*R* configuration for **3** which is the same 6′-*R* in the natural product **1**. To determine the C-3′ absolute configuration of **1** we used the 3,6-di-Mosher ester derivatives **5a** and **5b**. In **4a** and **4b** we observed that the *R*-, *S*-MTPA derivatization had little influence on the methoxy group chemical shift. Therefore, the negative $\Delta\delta^{SR}$ values

Table 3. Partial ^{1}H NMR (500 MHz, $CDCl_3$) data of the (*S*)- and (*R*)-Mosher ester derivatives **4a**, **4b**, **5a** and **5b**[a]

Hydrogen	δ_H		$\Delta\delta_{S-R}$	δ_H		$\Delta\delta_{S-R}$
	4a	**4b**		**5a**	**5b**	
$\underline{CH_3}$O-C1	3.72	3.72	0.00	3.59	3.66	−0.07
2	2.34	2.40	−0.06	2.44	2.55	−0.11
	2.42	2.48	−0.06	2.55	2.67	−0.12
3	3.88	3.97	−0.09	5.36	5.42	−0.06
6	5.09	5.09	0.00	5.04	4.96	+0.08

[a] Chemical shifts are referenced to TMS (δ 0.00).

observed in **5a** and **5b**[16] were assigned to the anisotropic influence of the ester group on C-3 (Table 3). For the hydrogens on C-2 (β-carbonyl) we had observed an effect which was taken into account in the absolute configuration of C-6 but in the 3,6-di-Mosher ester derivatives, **5a** and **5b** the values were 50% higher, which allowed a straightforward evaluation of the anisotropic effect arising from the Mosher acid group on C-3. From these observations we suggest a 3-*R* absolute configuration for compound **3**, which corresponds to a 3′-*R* configuration in **1**. Consequently oncidinol **1** is (2*S*,3′*R*,6′*R*)-1-acetyl-2-[3′,6′-diacetoxyeicosanyl)-glycerol.

In conclusion, this is the first report of a novel fully characterized diacylglycerol from *Ornithofora radicans* a major component of the secreted floral oil, which acts as a reward for pollinating bees. This paper is an example of mutualistic interactions in pollination and in science linking flower to bees and biologists to chemists.

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7. **Compound 2**. colorless oil, ^{1}H NMR (500 MHz, $CDCl_3$, δ_{TMS}) δ: 5.24 (m, 1H, H-3′), 4.85 (m, 1H, H-6′), 4.11 (m, 5H, H-1, H-2, H-3), 2.60 (m, 2H, H-2′), 2.10 (s, 3H, $\underline{CH_3}$CO-C1), 2.05 (s, 3H, $\underline{CH_3}$CO-C3′), 2.04 (s, 3H, $\underline{CH_3}$CO-C6′), 1.62 (m, 2H, H-4′), 1.53 (m, 2H, H-5′), 1.25 (m, 26H, H-7′–H-19′), 0.88 (t, J=6.7 Hz, 3H, H-20′).
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9. **Compound 1a**. *t*-Butyldimethylsilyl choride (34 mg, 0.23 mmol) was added to a stirred solution of a mixture **1** and **2** (180 mg in 0.5 mL of CH_2Cl_2), triethylamine (30 μL) and 4-dimethylaminopyridine (0.3 mg, 0.02 mmol). The mixture was further stirred at room temperature for 2 hours. The reaction was quenched with brine and extracted with ethyl acetate. The organic layer was treated with sodium sulfate and the organic solvent was evaporated under vacum producing a residue which was submitted to silica column chromatography eluting with hexane/ethyl acetate (1:1), producing 11 mg of **1a** (7% yield) and 120 mg of **2**.[7] Compound **1a**: colorless oil, ESMS 659 [M+H], $[\alpha]_D^{20}$ +16.5 (c=2.0, hexane). ^{1}H NMR (500 MHz, $CDCl_3$, δ_{TMS}) δ: 5.14 (m, 1H, H-3′), 5.00 (m, 1H, H-2), 4.78 (m, 1H, H-6′), 4.27 (dd, J=8.4 Hz, J=11.9 Hz, 1H, H-3), 4.08 (dd, J=5.9 Hz, J=11.9 Hz, 1H, H-3), 3.65 (d, J=5.1 Hz, 2H, H-1), 2.52 (m, 2H, H-2′), 2.01 (s, 3H, $\underline{CH_3}$CO-C1), 1.98 (s, 3H, $\underline{CH_3}$CO-C3′), 1.97 (s, 3H, $\underline{CH_3}$CO-C6′), 1.55 (m, 2H, H-4′), 1.46 (m. 2H, H-5′), 1.27 (m, 26H, H-7′-H-19′), 0.82 (m, 18H, H-20′ and CH_3 of TBDMS), ^{13}C NMR (124 MHz, $CDCl_3$, δ $CDCl_3$) δ: 170.65, 170.43, 170.02, 73.97, 72.21, 70.09, 62.63, 61.32, 39.13, 33.91, 32.01, 29.75, 29.69, 29.46, 25.87, 25.85, 25.42, 21.36, 21.14, 21.08, 22.81, 20.89, 14.26, −5.3.
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11. **Preparation of (*S*)- and (*R*)-MTPA ester derivatives of compound 2**. To a solution of **2** (9 mg, 0.0165 mmol) in CH_2Cl_2 (3mL), were sequentially added *S*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) (10 mg, 0.042 mmol), 4-dimethylaminopyridine (4 mg, 0.032 mmol) and 1,3-dicyclohexylcarbodiimide (8.7 mg, 0.042 mmol) at 0°C. The mixture was refluxed for 5 h under N_2 and then passed through a silica gel column eluting with CH_2Cl_2. The solvent was removed in vacuo, to give the *S*-Mosher ester **2a** (9.5 mg, 72% yield). Treatment with *R*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid as described above yielded the Mosher ester **2b** (9.0 mg, 68% yield). (^{1}H NMR data in Table 2).
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14. **Compound 3**. A solution of **2** (0.050 g, 0.091 mmol) in THF/H_2O/MeOH (3:1:1, 3.2 mL) and LiOH.H_2O (51 mg, 1.22 mmol) was stirred at 0°C for 30 min, the reaction was further stirred at room temperature overnight. The reaction mixture was neutralized to pH=7 with AcOH and extracted with $CHCl_3$. Evaporation of the solvent in vacuo furnished a crude residue (27.6 mg), which was methylated with diazomethane and purified by silica gel

column chromatography, eluting with hexane/ethyl acetate (1:1), to give **3** as a white solid (11.8 mg, 36% yield). (^{1}H NMR (500 MHz, $CDCl_3$, δ_{TMS}) δ: 4.02 (bs, 1H, H-3), 3.71 (s, 3H, $\underline{CH_3}$O-C1), 3.60 (bs, 1H, H-6), 2.53 (dd, $J=3.3$ Hz, $J=16.5$ Hz, 1H, H-2), 2.42 (dd, $J=8.4$ Hz, $J=16.5$ Hz, 1H, H-2), 1.56 (m, 2H, H-4), 1.43 (m, 2H, H-5), 1.27 (m, 26H, H-7–H-19), 0.88 (t, $J=6.2$ Hz, 3H, H-20).

15. **Preparation of mono-(*S*)- and (*R*)-MTPA ester derivatives 4a and 4b**. To a solution of **3** (5 mg, 0.022 mmol in CH_2Cl_2 (2 mL), were added sequentially *S*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (4.5 mg, 0.022 mmols), 4-dimethylaminopyridine (1 mg, 0.0081 mmol) and 1,3-dicyclohexylcarbodiimide (4.5 mg, 0.022 mmol) at 0°C. The mixture was stirred for 24 h under N_2 and then passed through a silica gel colunm eluting with hexane/ethyl acetate (8:1). The solvent was removed in vacuo, to give the mono-*S*-Mosher ester **4a** (1.1 mg, 14% yield). Treatment with *R*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid as described above yielded the mono-*R*-Mosher ester **4b** (0.9 mg, 11% yield). (^{1}H NMR data in Table 3).
16. **Preparation of di-(*S*)- and (*R*)-MTPA ester derivatives 5a and 5b**. A similar method to that described in Ref. 15 was adopted compound **3** (2 mg, 0.008 mmol in CH_2Cl_2) was sterified using 4 equivalents of *S*-(−)-MTPA (8 mg, 0.032 mmol), 4-dimethylaminopyridine (2 mg, 0.016 mmol) and 1,3-dicyclohexylcarbodiimide (7 mg, 0.032 mmol) at 0°C. The mixture was refluxed for 24 h under N_2 to yield di-*S*-Mosher ester **5a** (1,0 mg, 22% yield). Treatment of **3** with *R*-(+)-MTPA as described above yielded the di-*R*-Mosher ester **5b** (0.9 mg, 19% yield). (^{1}H NMR data in Table 3).