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First 6, 7-*Seco*-Clerodane Furan Diterpenoid from *Croton morifolius*

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Abstract: A novel 6,7-*seco*-clerodane furan-diterpenoid, named as Morifolin A, was obtained from *Croton morifolius*. The molecular structure of Morifolin A was determined by 1D/2D NMR spectroscopy, mass spectrometry and single-crystal X-ray diffraction analysis (Cu Kα). Morifolin A contains one furan ring bridged to a cyclohexene ring via an alkenyl chain and its crystal packing is displaying intermolecular interactions as C−H···O.

Keywords: *Croton morifolius;* 6,7 *seco*-clerodane furan-diterpenoid*;* morifolin A; absolute configuration; X-ray diffraction.

1. Introduction

The genus *Croton* (Euphorbiaceae) is a rich source of diterpenoids, such as clerodanes, tiglanes, kaurenes, labdanes, halimanes, cembranes, and pimaranes.[1] The structural diversity of clerodanes has been an important factor for a wide range of biological activities, such as: cytotoxic,[2–7] anti-inflamatory, [8–10] anti-microbial,[7, 11–12] anti-peptic ulcer[13] and NGF-potentiating.[14,15] Clerodane diterpenes are interesting for both natural products chemists and pharmacologists due to their biosynthetic significance, structural diversity and bioactivity.[16] In recent years, furan-clerodane diterpenoids,[1] which include a variety of fragments, such as: 3,4 epoxy,[5, 15, 17] 5,20 *δ*–lactone,

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[18] 9,12 and 19,20 γ–lactone,[18,19] 17,12 *δ*–lactone rings,[7,20–22] and a rare bicycle[5.4.0]undecane [23] have been isolated.

During a continuous search for phytochemical compounds from plants of Mexico, *Croton morifolius* was selected as a target to perform this phytochemical study by us. In this context, *C. morifolius*, also known as "palillo" or "vara blanca", is a shrub widely distributed in south central Mexico, which has been commonly used in traditional and ancestral medicine by locals who use it to treat various diseases such as: tonsillitis, gastritis, neuralgia, and stomach cancer, through an empirical treatment. Here, we report that roots of *C. morifolius* contains a new *seco*-clerodane furan diterpenoid, named Morifolin A (Figure 1). The absolute configuration of Morifolin A was confirmed by single-crystal X-ray diffraction studies.

2. Materials and Methods

2.1. General Experimental Procedures

Melting point was measured in a Mel-Temp II instrument and are uncorrected. Optical rotation was determined in $CHCl_3$ on a Perkin-Elmer 341 polarimeter. IR spectrum (Perkin–Elmer 2000 FTIR) was recorded (4000–400 cm^{-1} range) as KBr pellet. NMR measurements, including COSY, NOESY, HSQC, and HMBC experiments, were performed and recorded at 20 °C in $CDCl_3$ at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Bruker Asced 400 spectrometer, and spectra were referenced with the residual protio-solvent or TMS. High-resolution mass spectrometry (HRMS) was obtained in the electron impact (EI) mode at 70 eV on JEOL GCMatell. Column chromatographic separations was carried out on Merck silica gel 60 (Aldrich, 230-400 mesh ASTM). Column fractions were monitored by TLC, and the spots were visualized by heating plates after spraying with $CeSO_4$ in 5% H_2SO_4.

2.2. Plant Material

Specimens of *Croton morifolius* were collected in "*Temascales, Municipio de Contepec (Michoacán, Mexico)*" on July 2020, and were identified by M.C. Manuel González Ledesma. A voucher specimen (R. Velázquez Jiménez 10) has been deposited at the "Herbarium del Centro de Investigaciones Biológicas" of the "Universidad Autónoma del Estado de Hidalgo", Mexico (HGOM).

2.3 Isolation and Spectral Data of Morifolin A

The roots of *C. morifolius* (600 g) were exhaustively extracted by maceration at room temperature with MeOH (2.5 L x 2) twice by 7 days. The extract was filtrated and concentrated under vacuum pressure to yield a brownish residue (19.5 g).

The MeOH crude extract (10.0 g) was fractionated by flash column chromatography over silica gel (120 g), using a *n*-hexane/acetone mixture (9:1, 7:1 and 1:1) and acetone as eluents. Fractions of 100 mL of each polarity were collected and monitored by TLC and analyzed by ^{1}H NMR spectroscopy generating ten fractions (F.I–X). The fractions F.I-II were analyzed by ^{1}H NMR spectroscopy, which indicated that fatty acids were isolated. From F.III (976 mg) it was obtained as colorless crystals that were recrystallized from CH_2Cl_2 to yield Morifolin A (25 mg).

Morifolin A: Colorless crystal; mp 89–91 °C; $[\alpha]_D^{20}$= + 180.4 (*c* 0.95, $CHCl_3$); IR (KBr) *ν* = 3132, 1721, 1712, 1358 and 757 cm^{-1}; ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data, see Table 1. EIHRMS $[M]^+$ *m/z* 344.1990 (calcd. For $C_{21}H_{28}O_4$, 344.1988).

3. Results and Discussion

3.1. Isolation and Structure Elucidation

By applying column chromatography separation on a methanolic extract from roots of *C. morifolius*, a new *seco*-clerodane furanditerpenoid, named Morifolin A, was isolated. The Morifolin A

was characterized as 6,7-*seco*-clerodane furan diterpenoid based on its spectroscopic features according to the following discussion.

Morifolin A was isolated as colorless crystals. Its molecular formula $C_{21}H_{26}O_3$ with eigth degrees of unsaturation was determined from NMR data and EIHRMS spectra which showed the molecular ion $[M + H]^+$ at *m/z* 344.1990 (calcd. For $C_{21}H_{28}O_4$ 344.1988) in the spectrum. IR spectrum showed a strong stretching frequency band for C=O at 1714 cm $^{-1}$ characteristic of an aldehyde carbonyl group and characteristic bands for a *β*-substituted furan ring at 1375, 1159 and 822 cm^{-1}, respectively. The ^{1}H NMR data (Table 1) showed a typical signal for aldehyde group at δ 9.22 (H-6), three aromatic protons of a furan-3-yl moiety (δ_H 7.38 s, 7.36 s and 6.48 s), two olefinic protons from a disubstituted alkene at δ_H 5.82 (H-11) and 6.14 (H-12), an olefinic proton (δ_H 5.75, d, *J*= 5.0 Hz) from a trisubstituted alkene, a signal for ester methyl group at δ_H 3.71 (H-21), three methyl groups in H-18, H-19 and H-20 (δ_H 1.41 s, 1.10 s and 1.08 s), and secondary methyl for H-7 (δ_H 0.99, d, *J*= 7.0 Hz), respectively. The ^{13}C NMR spectrum displayed resonances of an additional ester carbonyl at δ_C 175.8 ppm, a disubstituted double bond at δ_C 133.1 and 120.1 ppm. The presence of the C-11 and C-12 double bond was supported by the ^{1}H-^{1}H COSY correlation of H-11/H-12 and HMBC correlations of H-11/C-10, H-12/C-14, H-12/C-16. The HMBC correlations of MeO-21/C-17 indicated that the methoxy group is positioned at C-17 (Figure 2a). NOESY correlations of H-10/H-6, H-10/Me-20, H-11/H-8 and Me-20/H-12, with the absence of correlation between H-10/Me 19 helped to assign the relative configuration (Figure 2b).

To assign the structure of Morifolin A, an X-ray diffraction experiment using graphite-monochromated Cu Kα radiation at 100 K was performed and yielded an absolute structure parameter of Flack, Parsons and Hooft parameters [0.01(18), 0.01(3) and 0.03(2)] and for inverted structure [1.00(18), 0.98(3) and 0.97(2)], respectively, which permitted assignment of the (5*R*, 8*S*, 9*R*, 10*R*) absolute configuration for Morifolin A (Figure 3). The above information suggested the presence of a $C_{6,7}$-*seco*-clerodane furan-diterpenoid, which is first *seco*-clerodane reported from *Croton* species to date.

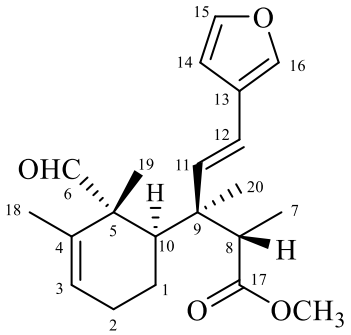

Figure 1. Chemical structure of morifolin A

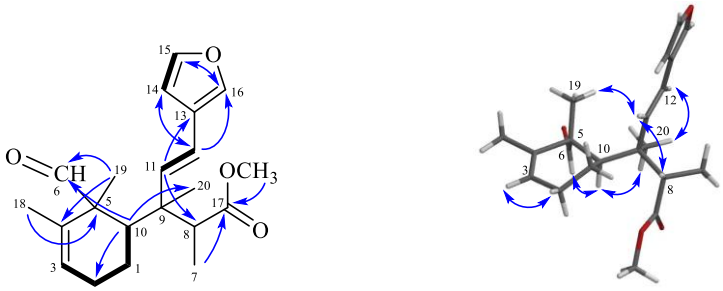

Figure 2. a) COSY and HMBC correlations and **b**) Key NOESY correlations of morifolin A

Table 1. ^{1}H and ^{13}C NMR data for Morifolin A in $CDCl_3$, (δ in ppm and *J* in Hz)[a]

Position	Morifolin A	
	δ_H, mult	δ_C
1	α 2.08, m[b]	20.0
	β 1.51, m	
2	α 2.06, m[b]	27.1
	β 2.14, m[b]	
3	5.75, d (5.0)	128.5
4	-	132.2
5	-	58.1
6	9.22, s	205.6
7	0.99, d (7.0)	12.8
8	2.89, q (7.0)	45.4
9	-	45.7
10	2.06, d (12.3)	47.6
11	5.82, d (16.2)	133.1
12	6.14, d (16.2)	120.1
13	-	124.3
14	6.48, s	107.3
15	7.38, s	143.5
16	7.36, br s	140.0
17	-	175.8
18	1.41, s	19.1
19	1.10, s	14.6
20	1.08, s	17.6
OMe-C-$_{17}$	3.71, s	51.5

[a]Assignments of ^{1}H and ^{13}C NMR data are based on $^{1}H-^{1}H$ COSY, HMQC, and HMBC experiments. [b]Signals are in overlapped regions of the spectra, and the multiplicities could not be discerned.

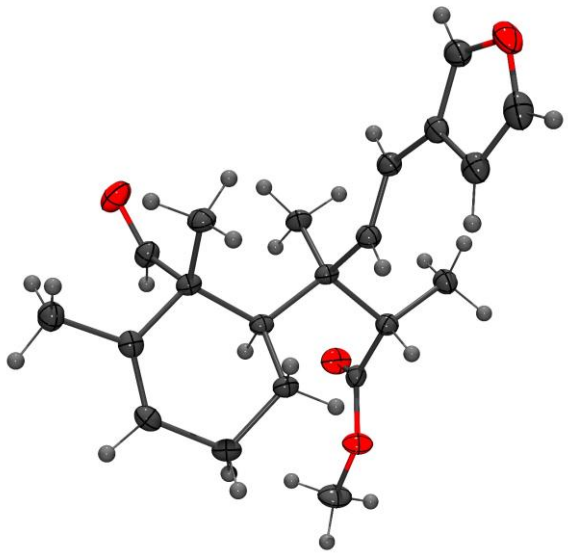

Figure 3. An ORTEP view of the molecular structure of Morifolin A. Ellipsoids are shown at the 50% probability level.

The packing of Morifolin **A** has a polymeric array via C–H···O interactions promoted by the oxygen atom of furan ring with a hydrogen atom from methylene of the six membered ring [C–H···O, d(H···O) = 2.530 Å; ∢(C–H···O) = 166.42°] and these polymeric aggregates are bridged together forming polymers via C–H···O interactions; a) from an oxygen atom of the aldehyde group with the hydrogen atom of the furan ring [C–H···O, d(H···O) = 2.793 Å; ∢(C–H···O) = 129.50°]; b) from an oxygen atom of the aldehyde group with the hydrogen atom of carbon atom with sp^2 hybridization [C–H···O, d(H···O) = 2.812 Å; ∢(C–H···O) = 146.87°] (Figure 4).

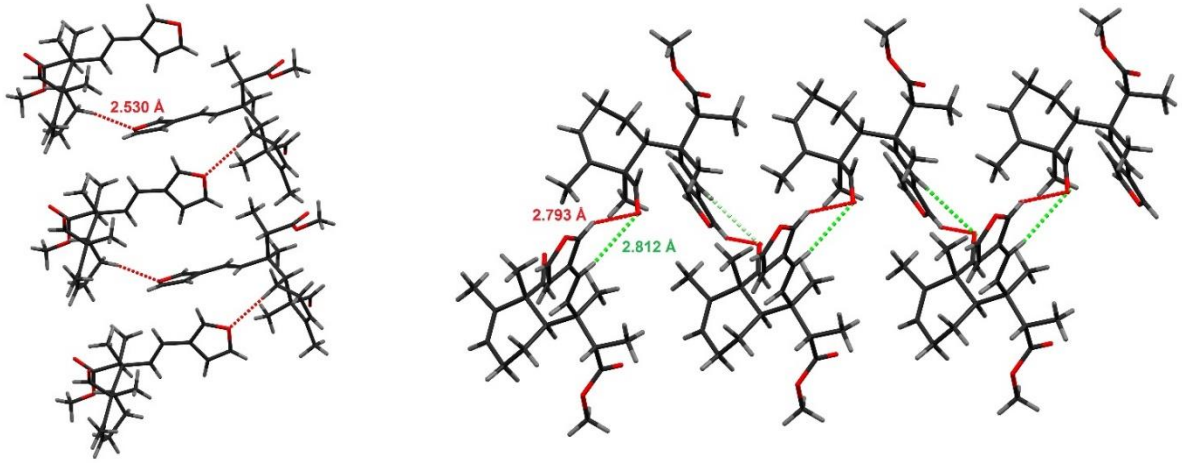

Figure 4. View of the C–H···O interactions present in the crystal structure of Morifolin A, (C, black; O, red; H, grey; Cg, red).

In summary, the species *Croton morifolius* contains a new *seco*-clerodane diterpenoid bearing a furan ring, which have been named as Morifolin A. To our knowledge there are no precedents of this type of diterpenoids in the literature then we propose the name of Morifolane for this new skeleton. The assignment of absolute configuration of Morifolin A was determined by X-ray diffraction analysis.

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X-ray Crystallographic Data

CCDC 2128619 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +441223 336033.

Supporting Information

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References

[1] W.H. Xu, W.Y. Liu and Q. Liang (2018). Chemical constituents from *Croton* species and their biological activities, *Molecules* **23**, 2333–2368.

[2] G. Wang, H. Zhang, H. Liu and J.M. Yue (2013). Laevinoids A and B: Two diterpenoids with an unprecedented backbone from *Croton laevigatus*, *J. Org. Lett.* **15**, 4880–4883.

[3] Q.Q. Yuan, S. Tang, W. Song, W.Q. Wang, M. Huang and L.J. Xuan (2017). Crassins A-H, diterpenoids from the roots of *Croton crassifolius, J. Nat. Prod.* **80**, 254–260.

[4] M. Qiu, D. Cao, Y. Gao, S. Li, J. Zhu, B. Yang, L. Zhou, Y. Zhou, J. Jin and Z. Zhao (2016). New clerodane diterpenoids from *Croton crassifolius*, *Fitoterapia* **108**, 81–86.

[5] K. Pudhom and D. Sommit (2011). Clerodane diterpenoids and a trisubstituted furan from *Croton oblongifolius, Phytochem. Lett.* **4**, 147–150.

[6] J.L.Tian, G.D. Yao, Y.X Wang, P.Y. Gao, D. Wang, L.Z. Li, B. Lin, X.X. Huang and S.J Song (2017). Cytotoxic clerodane diterpenoids from *Croton crassifolius*. *Bioorg. Med. Chem.* **27**, 1237–1242.

[7] C. Liu, J. Xu, J. Zhao, C. Xu, L. Dong, J. Ding and J. Yue (2014). Diterpenoids from *Croton laui* and their cytotoxic and antimicrobial activities, *J. Nat. Prod.* **77**, 1013–1020.

[8] K.W. Cordeiro, J.L. Felipe, K. F. Malange, P.R. Do Prado, P.de O Figueiredo, R.F. Garcez, K.D.C. Freitas and M.C. Toffoli Kadri (2016). Anti-inflammatory and antinociceptive activities of *Croton urucurana* Baillon bark, *J. Ethnopharmacol.* **183**, 128–135.

[9] P. Kuo, Y. Shen, M. Yang, S. Wang, T.D. Thang and N.X. Dung (2007). Crotonkinins A and B and related diterpenoids from *Croton tonkinensis* as anti-inflammatory and antitumor agents, *J. Nat. Prod.* **70**, 1906–1909.

[10] X. Su, Q. Liang, J. Hu, X. Zhang, R. Jia and W. Xu (2021). Diterpenoids from the whole plants of *Croton yunnanensis* and their bioactivities. *Bioorg. Med. Chem.* **51**, 116495.

[11] O. Rajachan, W. Lakornwong, S. Pitchuanchom, N.P. Suchaichit, J. Boonmak, S. Youngme, K. Kanokmedhakul and S. Kanokmedhakul (2021). *ent*-Clerodane diterpenoids from the stems of *Croton krabas*, *Fitoterapia* **152**, 104912.

[12] G. Wang, J. Li, G. Li, J. Xu, X. Wu, W. Ye and Y. Li (2012). Clerodane diterpenoids from *Croton crassifolius, J. Nat. Prod.* **75**, 2188–2192.

[13] E. Kitazawa, A. Sato, S. Takahashi, H. Kumano and A. Ogiso (1980). Novel diterpenlactones with anti-peptic ulcer activity from *Croton sublyratus*, *Chem. Pharm. Bull.* **28**, 227–234.

[14] Z. Pan, D. Ning, X. Wu, S. Huang, D. Li and S. Lv (2015). New clerodane diterpenoids from the twigs and leaves of *Croton euryphyllus, Bioorg. Med. Chem. Lett.* **25**, 1329–1332.

[15] Y. Sun, M. Wang, Q. Ren, S. Li, J. Xu, Y. Ohizumi, C. Xie, D. Jin and Y. Guo (2014). Two novel clerodane diterpenenes with NGF-potentiating activities from the twigs of *Croton yanhuii*, *Fitoterapia* **95**, 229–233.

[16] R. Li, S.L. Morris-Natschke and K. Lee (2016). Clerodane diterpenes: sources, structures, and biological activities, *Nat. Prod. Rep.* **33**, 1166–1226.

[17] R. Velázquez-Jiménez, D. Vargas-Mendoza, J.A. Gayosso-de-Lucio, S. González-Montiel and J.R. Villagómez-Ibarra (2018). Three novel epoxy-clerodanes bearing a furan ring from *Croton hypoleucus*, *Phytochem. Lett.* **24**, 21–26.

[18] M.K. Langat, B.M. Ndunda, C. Salter, B.O. Odusina, S.M. Isyaka, E. Mas-Claret, P.A. Onocha, J.O. Midiwo,J. Nuzillard and D.A. Mulholland (2020). Diterpenoids from the stem bark of *Croton megalocarpoides*, *Phytochem. Lett*. **39**, 1–7.

[19] B. Ndunda, M.K. Langat, H. Aaestman, M.R. Jacob, S.I. Khan, L.A. Walker, I. Mahammad, L.O. Kerubo and J.O. Midiwo (2016). New *ent*-clerodane and abietane diterpenoids from the roots of Kenyan *Croton megalocarpoides*, *Planta Med.* **82**, 1079–1086.

[20] J. Zhang, Y. Tang, J. Huang, W. Li, Y. Zou and G. Tang (2017). Bioactive diterpenoids from *Croton laevigatus*, *Phytochemistry* **144**, 151–158.

[21] M.G. Pizzolatti,A.J. Bortoluzzi, I.M.C. Brighente, A. Zuchinalli,F.K. Carvalho, A.C.S. Candido and M.T.L.P. Peres (2013). Clerodane diterpenes from bark of *Croton urucurana* Baillon, *J. Braz. Chem. Soc.* **24**, 609–614.

[22] D. S. B. Brasil, A.H. Müller, G.M.S.P. Guilhon, G. Peris, R. Llusar and V. Moliner (2010). Isolation, x-ray crystal structure and theoretical calculations of the new compound 8-epicordatin and identification of others terpenes and steroids from the bark and leaves of *Croton palanostigma* Klotzsch, *J. Braz. Chem. Soc.* **21**, 731–739.

[23] K. Siems, X.A. Dominguez and J. Jakupovic (1992). Diterpenes and other constituents from *Croton cortesianus*, *Phytochemistry* **31**, 4363-4365.

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