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Constituents of Organic Extracts of *Cuphea hyssopifolia*

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Abstract. From the aerial part of *Cuphea hyssopifolia* (Lythraceae) diterpenes and flavonoids were isolated. The compounds were identified as: friedelan-3β-ol **1**, ursolic acid **2**, methyl gallate **3**, quercetin **4**, quercetin-3-*O*-α-rhamnopyranoside **5**, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose **6** and manitol **7**.
Keywords: *Cuphea*, Lythraceae, flavonoid, triterpene, NMR.

Resumen. Diterpenos y flavonoides fueron aislados a partir de la parte aérea de *Cuphea hyssopifolia* (Lythraceae). Los compuestos fueron identificados como: friedelan-3β-ol **1**, ácido ursólico **2**, galato de metilo **3**, quercetina **4**, quercetina-3-*O*-α-ramnopiranósido **5**, 1,2,3,4,6-penta-*O*-galoil-β-D-glucosa **6** y manitol **7**.
Palabras clave: *Cuphea*, Lythraceae, flavonoide, triterpenos, RMN.

Introduction

The genus *Cuphea* (Lythraceae) includes about 250 herbaceous or sub-shrubby species ranging from the United States to Argentina [1]. Some species of the genus *Cuphea* have been used for treating stomach disorders, syphilis, gonorrhea, cancer, as well as an oral contra-ceptive in folk medicines from South and Central Americas [2-5]. As part of our interest in folk medicine from the state of Oaxaca Mexico, we have undertaken the analysis of a Mexican *Cuphea*. Thus, in this paper we describe the structure elucidation of diterpenes and flavonoids, isolated from ethyl acetate and methanolic extracts of the aerial parts of *Cuphea hyssopifolia* (Humb. Bompl. et Kunth), which was collected in Santa Maria Tlahuitoltepec Mixe, small town 123 Km from Oaxaca city. In this town, infusions of the aerial parts of *Cuphea hyssopifolium* are used for treating stomach disorders [6]. In 1999 Yang *et al*. [7] reported the isolation of seven hydrolysable tannins including two new dimeric ellagitannins with macrocyclic structures from the aqueous extract of *Cuphea hyssopifolium,* which was collected in Taipei, Taiwan. The macrocyclic structures showed activity both in vitro and in vivo on human promyeolocitic leukemia (HL-60) cells [8,9]. However, the study of the organic extract was not reported by these authors. Thus, we considerate opportune to study the organic extract of *Cuphea hyssopifolium,* with the idea of complement the previous study.

Results and Discussion

The aerial part of *Cuphea hyssopifolia* was dried, ground and extracted consecutively with hexane, ethyl acetate and methanol, at room temperature in a closed container several times. The extracts were concentrated under reduced pressure at 40 °C yielding three different extracts. After removal of solvent, the hexane extract was fractionated on a silica gel column, eluted with a gradient hexane: ethyl acetate (9:1→1:9) yielding 9 fractions (IA-IXA). From the first refined fraction (IA) 30 mg of friedelan-3β-ol **1** were isolated, while the third fraction (IIIA) furnished 55 mg of ursolic acid **2** (Figure 1). Both products were identified by means of NMR and comparison with the data previously reported in the literature [10,11]. To our delight, the ethyl acetate extract resulted more interesting because was possible to isolate two flavonoids and an aromatic compound. Thus, the ethyl acetate extract was fractionated on a silica gel column, eluting with a hexane-ethyl acetate (9:1→1:9) gradient yielding 9 fractions (IB-IXB). 120 mg of methyl gallate **3** were isolated from the fraccion IVB. Quercetin **4** (30 mg) and quercetin-3-*O*-α-rhamnopyranoside **5** (33 mg) were isolated from the fractions VIB and VIII respectively. It was possible to find in the methanolic extract 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose **6** (20 mg), which has been isolated from the aqueous extract of the same plant [7]. The methanolic extract was fractionated on a flash silica gel column, eluted with hexane:ethyl acetate (2:8→0:10) and ethyl acetate:methanol (9:1→8:2) gradient to give 5 fractions (IC-VC). Mannitol precipitates as white solid (1.2 g) from the fraction VC.

In a preview phytochemical study of *Cuphea wrihtii* reported by Perez-Castorena [12] and Maldonado, friedelan-3β-ol **1** and ursolic acid **2** were identified as constituents of the acetonic extract. Quercetin **4** is a flavonid present in *C. diosmifolia*, *C. pseudovaccinium*, *C. sclerophylla* and *C. Crulsiana* [13]. The flavonoid quercetin-3-*O*-α-rhamnopyranoside **5,** was also found in *C. pinetorum* [14]. Mannitol has been isolated from *C. wrihtii*, *C. pseudovaccinium*, *C. sclerophylla* and *C. Crulsiana* [11]. With this information, it is possible to establish an important chemical composition relationship, between *Cuphea hyssopifolia* and the other *Cuphea* species. Additionally, during the development of this work, it was possible to isolate the methyl gallate **3**, which has not been reported in other *Cuphea* specie. However, the presence of that compound cannot be employed as a taxonomic tool for distinction between *Cuphea*

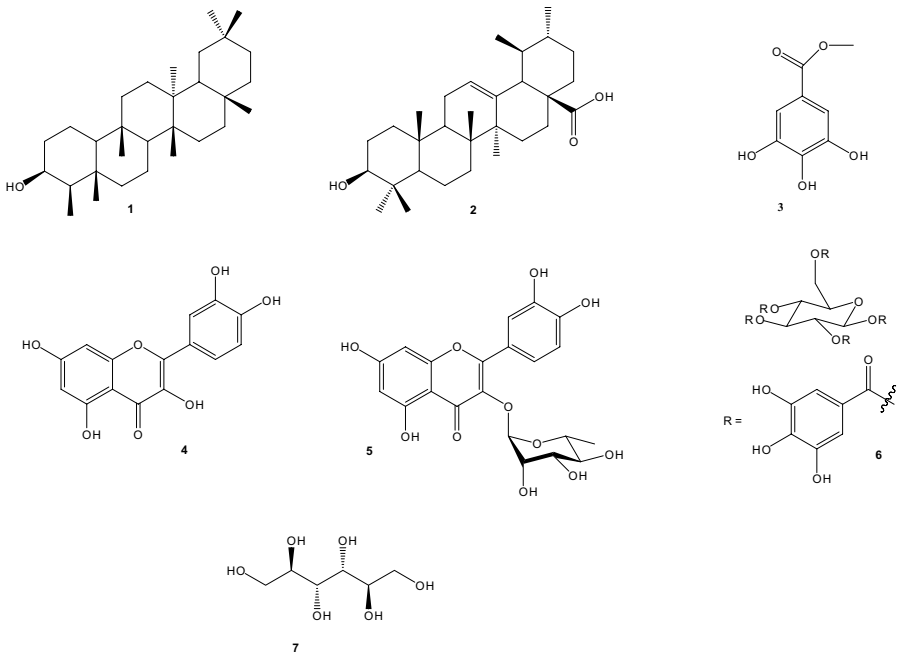

Figure 1. Metabolites isolated from organic extract of *Cuphea hyssopifolia*.

species, once the ester **3** could be product of work conditions. In the future, we will try to clarify this unknown, and to be able to confirm that ester **3** is a chemical marker.

Conclusions

In summary, we were able to isolate seven known compounds from the organic extract of *Cuphea hyssopifolia*. These compounds allowed us to establish a relationship between different *Cuphea* species. Additionally, this is the first time that the compound **3** has been isolated from the genus *Cuphea*. Finally, is important to remark that, the value of the present study lies in the importance of the plant to the folk medicine in the state of Oaxaca.

Experimental Part

General experimental procedures. Infrared spectra were recorded as pellet/KBr with a Fourier transform (FTIR) Nicolet FT-IR 750 spectrometer. Mass spectra were recorded with a Jeol JMS-SX505 and Jeol JMS-102 high-resolution mass spectrometers. NMR experiments were conducted on a Varian 500 MHz instrument using $CDCl_3$ CD_3OD and D_2O (99.9% D) as the solvent, with chemical shifts (δ) referenced to internal standards $CDCl_3$ (7.26 ppm ^1H, 77.0 ppm ^{13}C) or Me_4Si as an internal reference (0.00 ppm). Chemical shifts are in parts per million (ppm). Column chromatography (CC) was performed using silica gel GF_{254} and flash silica gel (230-400) and employed a solvent polarity correlated with TLC mobility. Developed TLC plates were visualized under a short-wave UV lamp and by heating plates that were dipped in $Ce_2(SO_4)_3$.

Plant material. *Cuphea hyssopifolia* was collected in Santa Maria Tlahuitoltepec Mixe, Oaxaca Mexico, in 1995 and identified by Dr. Francisco Ramos from the Institute of Biology of the UNAM (Institution Code IBUNAM Collection Code MEXU Catalog Number OAX462546).

Extraction and Isolation. Dried and powdered aerial parts of *C. hyssopifola* (800 g) were extracted with hexane for 48 h (12 L) three times, at room temperature in a closed container. The combined extracts were concentrated under reduced pressure at 40 °C to give a viscous concentrate (8.5 g). In a second stage, the solid residue was extracted with EtOAc (12 L) three times for 48 h, at room temperature in a closed container. Later of solvent elimination 15 g of a viscous concentrate were obtained. Finally, the solid residue was extracted with methanol (12 L) three times for 48 h, at room temperature in a closed container, to give a viscous concentrate (30 g)

The hexanic extract was fractionated on a silica gel column (GF_{254}), eluted with a gradient hexane: ethyl acetate (9:1→1:9) yielding 10 fractions (IA-IXA). The friedelan-3β-ol **1** (30 mg) was obtained from the fraction IA by flash chromatography. From fraction IIIA was isolated ursolic acid **2** (55 mg).

The residue obtained with AcOEt (13.5 g) was dissolved in CH_2Cl_2, celite was added, and after the solvent had been removed in vacuo, the extract was subjected to silica gel chromatography eluting with a gradient hexane:ethyl acetate (9:1→ 1:9) to give 9 fractions (IB-IXB). The methyl gallate **3** (120 mg) was obtained from the fraction IVB by flash chromatography. From fractions VI and VIII quercetin **4** (30 mg) and quercetin-3-*O*-α-rhamnopyranoside **5** (33 mg) were isolated.

Finally, the methanolic extract was subject to purification by means of column chromatography, using flash column, eluted with hexane:ethyl acetate (2:8→0:10) and ethyl acetate: methanol (9:1→8:2) to give 5 fractions (IC-VC). Extensive

purification by column chromatography of fraction IVC led to the isolation of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose **6** (10 mg). Mannitol precipitates as white solid (1.2 g) from the fraction VC.

Friedelan-3β-ol 1: mp 287-289 °C (Lit. [15] 286-289 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.7 (1H, m), 1.58-1.54 (bunch for 24H), 1.0 (3H, s), 0.99 (6H, s), 0.96 (3H, s), 0.94 (3H, s), 0.93 (3H, d, *J* = 5.5 Hz), 0.86 (3H, s). ^{13}C NMR (75 MHz, CH_3OD): δ 72.7, 61.3, 53.1, 49.1, 42.8, 41.7, 39.6, 39.2, 38.3, 37.8, 37.1, 36.1, 35.5, 35.3, 35.1, 35, 32.8. 32.3, 32, 31.8, 30.6, 30, 28.2, 20.1, 18.6, 18.2, 17.5, 16.4, 15.8, 11.6. IR (pellet/KBr): 3480, 2925, 1580, 1460, 1390, 1002, 1001 cm^{-1}. EI-MS (*m/z*): 428.

Ursolic acid 2: mp 237-239 °C (Lit. [11] 237-240 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.40 (1H, m), 3.20 (1H, dd, *J* = 11.7, 4.5 Hz), 2.13 (1H, d, *J* = 11.3 Hz), 1.96 (1H, td, *J* = 13.4, 4.9 Hz), 1.86 (2H, dd, *J* = 12.6, 7.3 Hz), 1.81 (1H, td, *J* = 13.6, 7.2 Hz), 1.67 (1H, td, *J* = 13.9, 6.8 Hz), 1.58 (4H, m), 1.47 (4H, m), 1.33 (3H, m), 1.21 (1H, m), 1.04 (3H, s), 0.98 (1H, m), 0.93 (4H, m), 0.90 (3H, d, *J* = 6.0 Hz), 0.87 (3H, s), 0.81 (3H, d, *J* = 6.4 Hz), 0.76 (4H, m), 0.73 (3H, s), 0.68 (1H, d, *J* = 11.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.1, 138.5, 125.8, 79.1, 55.5, 53.1, 48.1, 47.8, 42.3, 39.7, 39.4, 39.2, 39, 38.9, 37.2, 37.1, 33.3, 30.9, 28.3, 27, 24.5, 23.8, 23.6, 21.5, 18.6, 17.3, 17.1, 15.9, 15.7. IR (pellet/KBr): 3562, 2947, 2866, 1697, 1460, 1385, 1306, 1030. EI-MS (*m/z*): 456.

Methyl gallate 3: mp: 198-200 °C (Lit. [16] 202 °C). ^{1}H NMR (300 MHz, CD_3OD): δ 7.03 (2H, s), 3.79 (3H, s). ^{13}C NMR (75 MHz, CD_3OD): δ 169, 146.4, 139.7, 121, 110, 52.2. IR (pellet/KBr): 3468, 33390, 1694, 1315, 1256, 1197. EI-MS (*m/z*): 184. The compound was identified after comparison with data previously reported in the literature [17].

Quercetin 4: mp: 313-315 °C (Lit. [18] 313-314 °C). ^{1}H NMR (300 MHz, CD_3OD): δ 7.71 (1H, d, *J* = 2.1 Hz), 7.61 (1H, dd, *J* = 8.5, 2.1 Hz), 6.86 (1H, d, *J* = 8.5 Hz), 6.36 (1H, d, *J* = 2 Hz), 6.16 (1H, d, *J* = 2 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 177, 166, 163, 158, 149, 148, 146, 137, 124, 121, 115.6, 115.3, 105, 99, 94. IR (pellet/KBr): 3388, 1657, 1612, 1245, 1165, 1093. EI-MS (*m/z*): 302. The compound was identified after comparison with data previously reported in the literature [19].

Quercetin-3-*O*-α-rhamnopyranoside 5: mp: 175-177 °C (Lit. [20] 179-182 °C). ^{1}H NMR (300 MHz, CD_3OD): δ 7.33 (1H, d, *J* = 2.1 Hz), 7.30 (1H, dd, *J* = 8.5, 2.1 Hz), 6.90 (1H, dd, *J* = 8.5 Hz), 6.35 (1H, d, *J* = 2 Hz), 6.19 (1H, d, *J* = 2 Hz), 5.34 (1H, d, *J* = 2 Hz), 4.20 (1H, dd, *J* = 8.5, 2.1 Hz), 3.76 (1H, d, *J* = 2.1 Hz), 3.76 (1H, d, *J* = 2.1 Hz), 3.33 (1H, m). ^{13}C NMR (75 MHz, CD_3OD): δ 178, 165.9, 163.2, 158.5, 149.8, 148, 146.4, 137, 122.8, 116.9, 116.3, 103.2, 101.9, 73.2, 72, 71.8, 17.6. IR (pellet/KBr): 3341, 2948, 1656, 1500, 1200. EI-MS (*m/z*): 450. The compound was identified after comparison with data previously reported in the literature [21].

1,2,3,4,6-penta-*O*-galloyl-β-D-glucose 6: ^{1}H NMR (300 MHz, CH_3OD): δ 7.11 (2H, s), 7.05 (2H, s), 6.97 (2H, s), 6.94 (2H, s), 6.89 (2H, s), 6.35 (1H, d, *J* = 8.2 Hz), 5.40 (1H, t, *J* = 9.0 Hz), 5.93 (1H, t, *J* = 9.6 Hz), 5.42 (1H, t, *J* = 9.7 Hz), 4.57 (1H, m), 4.29 (1H, brs), 3.74 (1H, m). NMR (75 MHz, CD_3OD): δ 167.9, 167.3, 167, 166.9, 166.2, 146.5, 146.4, 146.3, 146.2, 140.8, 140.3, 140.2, 140.1, 139.9, 121.1, 120.4, 120.3, 120.2, 119.8, 110.6, 110.5, 110.4, 110.4, 110.3, 93.8, 74.4, 74.1, 72.2, 69.8, 63.1. IR (pellet/KBr): 3394, 1701, 1615, 1536, 1450, 1321, 1213, 1209, 1033, 960, 872, 805. EI-MS (*m/z*): 940. The compound was identified after comparison with data previously reported in the literature [17].

Mannitol 7: mp: 169-171 °C (Lit. [22] 168-170 °C). ^{1}H NMR (300 MHz, D_2O): δ 3.6 (8H, m). ^{13}C NMR (75 MHz, D_2O): δ 70.8, 69.2, 63.2. IR (pellet/KBr): 3341, 2948, 1656, 1500, 1200. EI-MS (*m/z*): 182.

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References

1. Amarasingue, V.; Graham, S. A.; Graham, A. *Bot. Gaz.* **1991**, *152*, 77-79.
2. Krepsky, P. B.; Farias, M. R.; Côrtes, S. F.; Braga, F. C. *Biochem. Syst. Ecol.* **2010**, *38*, 125-127.
3. Martins, D.; Roque, N. F. *Fitoterapia*, **1995**, *66*, 187.
4. Gonzalez, A. G.; Valencia, E.; Siverio Exposito, T.; Bermejo Barrera, J.; Gupta, M. P. *Planta Med.* **1994**, *60*, 592-593.
5. Elisabetsky, E.; Posey, A. D. *J. Ethnopharm.* **1989**, *26*, 299-316.
6. Oral communication from Santa Maria Tlahuitoltepec Mixe, Oaxaca México natives.
7. Chen, L. G.; Yen, K. Y.; Yang, L. L.; Hatano, T.; Okuda, T.; Yoshida, T. *Phytochemistry*, **1999**, *50*, 307-312.
8. Wang, C. C.; Chen, L. G.; Yang, L. L. *Cancer Lett.* **2000**, *149*, 77-83.
9. Wang, C. C.; Chen, L. G.; Yang, L. L. *Cancer Lett*. **1999**, *140*, 195-200.
10. Kumar, S.; Raj, K.; Khare, P. *Indian J. Chem. Sec. B*, **2009**, *48B*, 291-294.
11. Gnoatto, S. C. B.; Dassonville-Klimpt, A.; Da Nascimento, S.; Galéra, P.; Boumediene, K.; Gosmann, G.; Sonnet, P.; Moslemi, S. *Eur. J. Med. Chem*. **2008**, *43*, 1865-1877.
12. Perez-Castorena, A. L.; Maldonado, E. *Biochem. Syst. Ecol*. **2003**, *31*, 331-334.
13. Santos, D. Y. A. C.; Salatino, M. L. F.; Salatino, A. *Biochem. Syst. Ecol*. **1995**. *23*, 99-103.
14. Calzada, F. *Phytother. Res.* **2005**, *19*, 725-727.
15. Zhida-Da, M.; Mizuno, M.; Tanaka, T.; Iinuma, M.; Guang-Yi, X.; Qing, H. *Phytochemistry*, **1989**, *28*, 553-1989.
16. Kumar, R. J.: Jyostna, D.; Krupadanam, G. L. D.; Srimannarayana, G. *Phytochemistry*, **1988**, *27*, 3625-3626.
17. Kiss, A. K.; Derwinska, M.; Dawidowska, A.; Naruszewicz, M. *J. Agric. Food Chem.* **2008,** *56,* 7845-7852
18. Yuldashev, M. P.; Muminova, B. A.; Drenin, A. A.; Botirov, E. Kh. *Chem. Nat. Compd.* **2007**, 43, 34-36.
19. Fossen, Torgils; Pedersen, A. T.; Andersen, O. M. *Phytochemistry*, **1997**, *47*, 281-285.
20. Sirat, H. M.; Rezali, M. F.; Ujang, Z. *J. Agric. Food Chem*. **2010**, *58*, 10404-10409.
21. Hanamura, T.; Hagiwara, T.; Kawagish, H. Biosci. Biotechnol. Biochem. 2005, 69, 280-286.
22. Sugihara, J. M.; Yuen, G. U. *J. Am. Chem. Soc*. **1957**, *79*, 5780-5782.