PERGAMON

Phytochemistry 52 (1999) 1159–1163

PHYTOCHEMISTRY

An unusual isopropenyldihydrofuran biflavanol from *Tephrosia crassifolia*☆

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Received 13 November 1998; received in revised form 30 April 1999

Abstract

The roots and aerial parts of *Tephrosia crassifolia* afforded an isopropenyldihydrofuran biflavanol and a new chalcone. The structures and relative configuration were established by spectroscopic and chemical methods, and confirmed by X-ray diffraction. Crassifolin is the second 3,4″ ether-linked biflavan-4-ol to be isolated from the genus *Tephrosia*. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Tephrosia crassifolia; Leguminosae; Isopropenyldihydrofuran biflavanol; (9; 10; 9″; 10″-dihydro-5; 5″-dimenthoxy-10; 10″-isopropenyl-dihydrofuran-(7; 8; 7″; 8″)-4; 4″-biflavanyl ether) crassifolin; (4″; 5″-dihydro-3′-hidroxy-6′-methoxy-isopropenyldihydrofuran(2″; 3″; 4′; 3′)-chalcone) crassichalcone

1. Introduction

In a continuation of our chemical studies of members of the genus *Tephrosia* (Leguminosae) (Gómez-Garibay, Quijano, García, Calderón & Ríos, 1983; Gómez-Garibay, Quijano, Calderón, Rodríguez, Rodríguez & Ríos, 1985a; Gómez-Garibay, Calderón, Quijano, Domínguez & Rios, 1985b; Gómez-Garibay, Quijano, Calderón, Morales & Ríos, 1988; Gómez-Garibay, Quijano & Ríos, 1991; Gómez-Garibay, Calderón, Quijano, Tellez, Olivares & Ríos, 1997), we have undertaken the study of *Tephrosia crassifolia*, a species endemic to the northwest of México. Isolation of the components of this plant afforded a novel ether-linked biflavan-4-ol and a novel chalcone. Most biflavonoids isolated from natural sources posses a C–C bridge with only a few having an ether bridge (Geiger, 1994). As far as we know flavan-4-ols and biflavan-4-ols are compounds with limited natural occurrence (Porter, 1988, 1994).

2. Results and discussion

Extraction of the roots and aerial parts of the plant with petrol, ethyl acetate and methanol, followed in each case by CC and preparation TLC over silica-gel (see Experimental) gave two new flavonoids which we named crassifolin (**1**) and crassichalcone (**2**). In addition, the known β-sitosterol, stigmasterol, and the flavonoids, abbottin (**3**) (Gómez-Garibay, Quijano, Calderón, Aguirre & Ríos, 1986), hildgardtol A (**4**) (Delle Monache, Labbiento, Marta & Lwande, 1986), methyl hildgardtol A (**5**) Delle Monache et al., 1986), 5-*O*-methylobovatin (**6**) (Gómez-Garibay et al., 1986), oaxacacin (**7**) (Domínguez, Téllez & Ramírez, 1983) and sucrose were isolated (Fig. 1). Identification of known compounds was based on comparison with authentic samples and published data.

Crassifolin A (**1**) was isolated as a crystalline pro-

☆ Part 9 in the series 'Flavonoids from *Tephrosia* Species'. For Part 8 see Gómez et al. (1991). Contribution No. 1693 of Instituto de Química, UNAM.

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0031-9422/99/$ - see front matter
PII: S0031-9422(99)00336-2

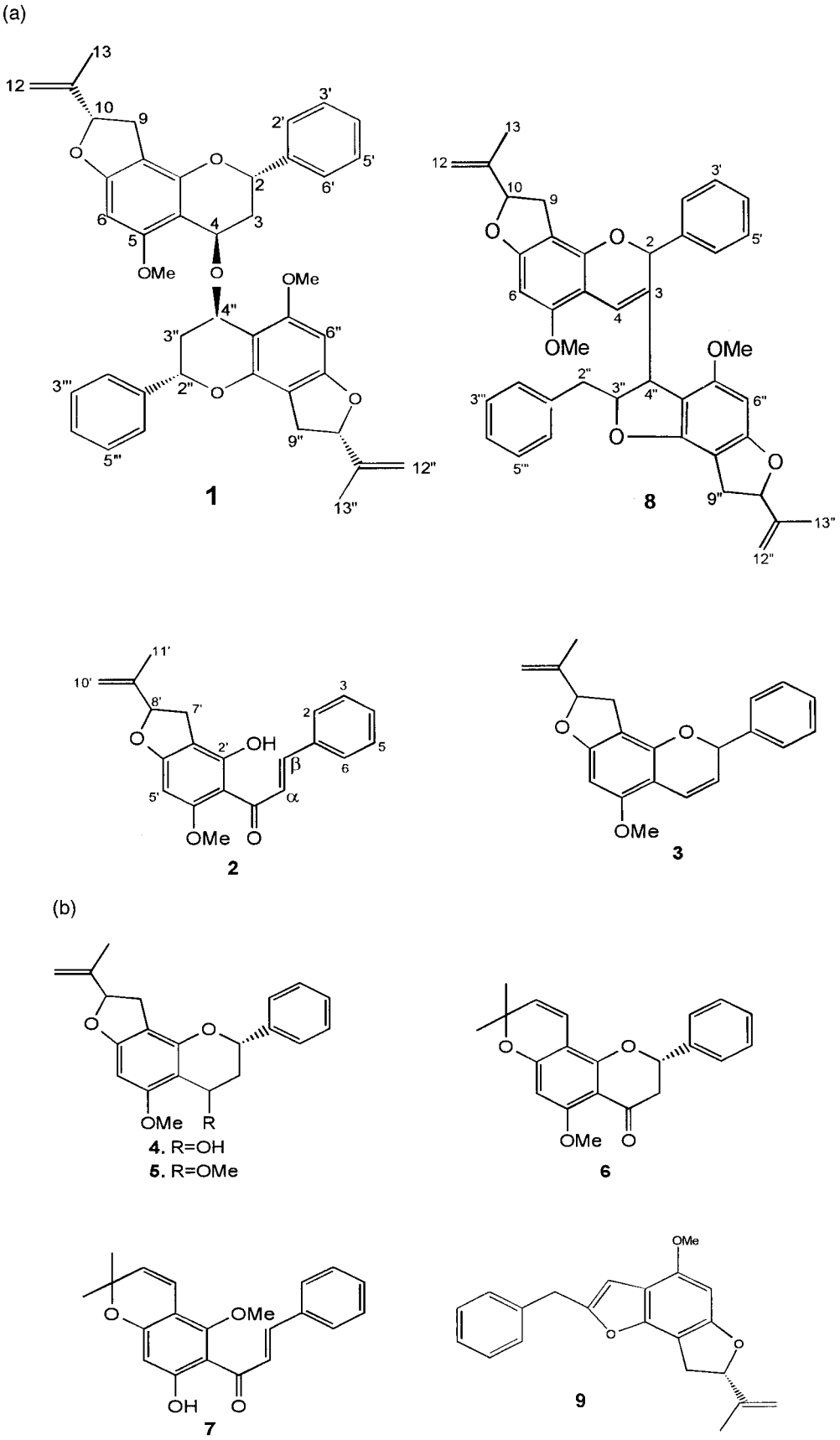

Fig. 1. Chemical structures of flavonoids.

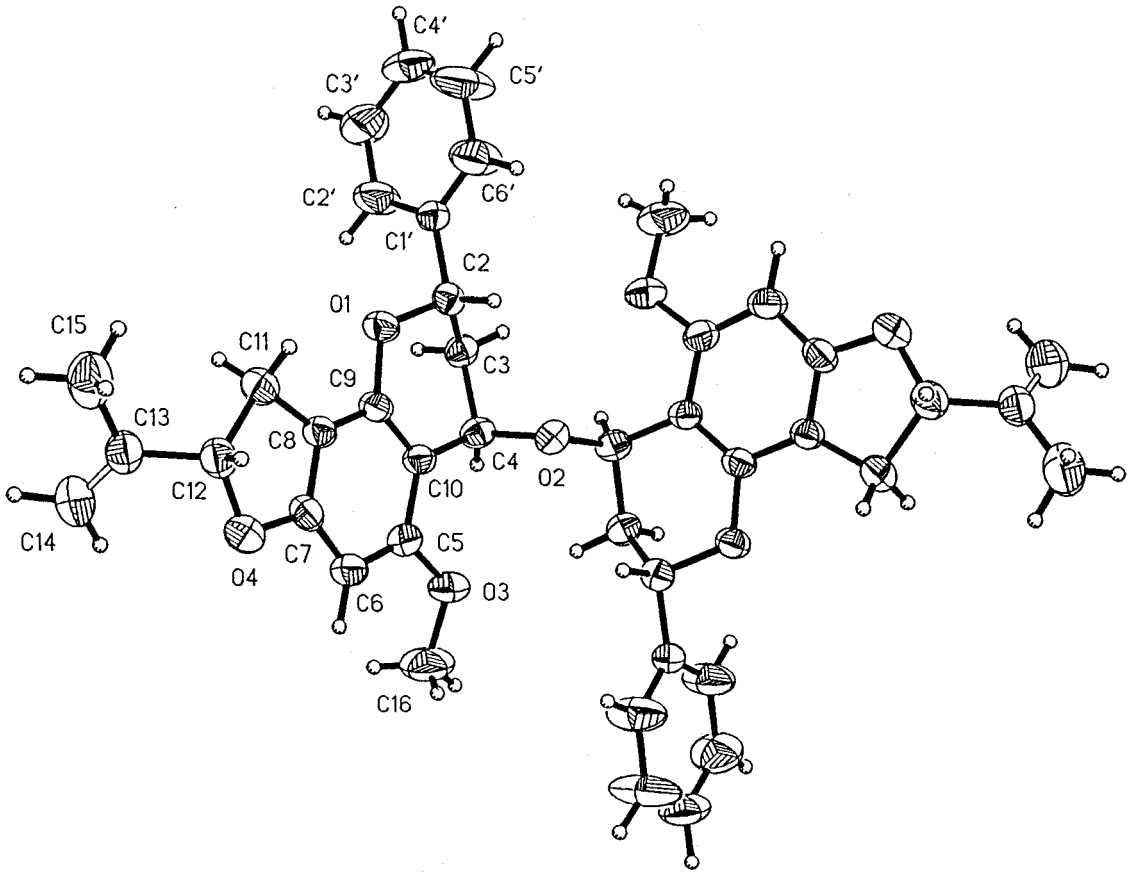

Fig. 2. Stereoscopic view of crassifolin A (**1**).

duct mp 249–250°. $[\alpha]_D$ +4.24 ($CHCl_3$), *c* (3.3). Its IR spectrum showed the presence of aromatic bands (1645 cm^{-1}) but lacked either any hydroxyl or carbonyl adsorption. The ^1H NMR spectrum of **1** closely resembled that of hildgardtol A (**4**) previously isolated from *T. hildebrandtii* (Delle Monache et al., 1986) except for the different chemical shift of H-4, which was shifted downfield to 4.9 ppm in crassifolin (**1**). The UV and MS spectra of crassifolin (**1**) suggested a biflavanol structure. The UV spectrum showed two maxima in the region of 248 and 279 nm, the magnitude of the molar extinction coefficients being almost double those for typical flavonoids such as hildgardtol A (**4**) (Delle Monache et al., 1986), thus confirming the above assumption.

The MS showed a molecular ion peak at *m/z* 658 corresponding to the molecular formula $C_{42}H_{42}O_7$, which is indicative of an isopropenylfuran biflavanol, and also some characteristic fragments at *m/z* 338 [M–$C_{21}H_{22}O_4$] and 321 [$C_{21}H_{21}O_3$] which are consistent with structure **1**. Confirmation of the structure and relative configuration of crassifolin A (**1**) was achieved by a single crystal X-ray analysis (Fig. 2).

Treatment of **1** with *p*-toluenesulfonic acid furnished abbottin (**3**) and the unexpected dimer **8**, which could be formed through the reaction between compound **3** and compound **9** (not isolated) under acidic conditions (Gómez-Garibay et al., 1997). This compound is a crystalline product mp 115–117°. Its molecular weight, determined by mass spectrometry, is in accord with the molecular formula $C_{42}H_{40}O_6$. The IR spectrum indicated the presence of aromatic groups (1621 cm^{-1}). Structure **8** followed from analysis of ^1H NMR signals together with mass spectral peaks. In the same way, by treatment of hildgardtol A (**4**) or methylhildgardtol A (**5**) with *p*-toluenesulfonic acid the same compounds were obtained.

Crassichalcone (**2**) was isolated as a yellow oil, $C_{21}H_{22}O_4$, $[M]^+$ 336. The ^1H NMR spectrum, δ 7.74 (1H, *d*, J = 15.8 Hz, H-α) and δ 7.85 (1H, *d*, J = 15.8 Hz, H-β) supported a chalcone structure with an isopropenyldihydrobensofuran residue δ 1.76 (3H, *s*, H-11′), δ 2.94 (1H, *dd*, J = 7.5, 15.0 Hz, H-7′ax), δ 3.29 (1H, *dd*, J = 9.9, 15.0 Hz, H-7′eq), a methoxyl group at δ 3.9 (3H, *s*, OMe), δ 4.92 (1H, *s*, H-10′a), δ 5.07 (1H, *s*, H-10′b), and at δ 5.28 (1H, *dd*, J = 9.9, 7.5 Hz, H-8′). The UV spectrum was typical of a chalcone (Domínguez, 1973). On the basis of this information and the mass spectrum fragmentation pattern, structure **2** was assigned.

3. Experimental

T. crassifolia was collected in Nayarit, México, Municipality of Compostela, 27 January 1995. A voucher specimen is deposited in the Herbarium of the Instituto de Biología (UNAM), México.

3.1. Extraction and separation

The air-dried leaves and stems (825 g) were extracted successively with petrol, EtOAc and MeOH. After solvent evapn green syrups A (5.3 g), B (12.1 g) and C (44.3 g) were obtained. In the same way, from the air-dried roots (810 g), green syrups D (16.3 g), E (27.3 g) and F (39.5 g) were obtained.

Extract A (5.3 g) was chromatographed on a silica gel column (300 g), eluting with petrol and mixts of petrol $-CH_2Cl_2$. From the frs eluted with petrol 18.3 mg of sitosterol–stigmasterol and 12 mg 5-*O*-methylobovatin (**6**) (mp 159–161°) were obtained.

Extract B (12.1 g) was fractionated on silica gel (500 g) using petrol and mixts of petrol $-CH_2Cl_2$ (8:2) and gave sistosterol–stigmasterol, 82 mg hildgardtol A (**4**) (Delle Monache et al., 1986) (mp 111–114°), 15.3 mg 5-*O*-methylobovatin (**6**), 12 mg abbottin (**3**) (Gómez-Garibay et al., 1986), 49 mg oaxacacin (**7**) (Domínguez et al., 1983) and 477 mg crassichalcone (**2**), identified by IR, ^{1}H NMR, EM and comparison with authentic samples.

Extract D (16.3 g) dissolved in hexane at ambient temperature afforded 6.3 g of hildgardtol A (**4**) (mp 111–114°). The mother liquor from D was chromatographed on a silica gel column (500 g), eluting with hexane, CH_2Cl_2 and mixtures of CH_2Cl_2–EtOAc. From frs eluted with hexane a mixt. of sitosterol–stigmasterol was obtained. From frs eluted with hexane-CH_2Cl_2 25 mg abbottin (**3**), 560 mg of hildgardtol A (**4**), and 242 mg of crassifolin (**1**) were obtained. In the same way, extract E (27.3 g) dissolved in hexane afforded 1.98 g of crassifolin (**1**) (mp 249–250°). The mother liquor from E was chromatographed on a silica gel column (600 g) affording 213 mg abbottin (**3**), 1.3 g hildgardtol A (**4**), 25 mg 5-*O*-methylobovatin (**6**), 1.47 g crassifolin (**1**) and 838 mg of methylhildgardtol A (**5**) (Domínguez et al., 1983) (mp 123–124).

Finally, from extract F (25.3 g), 8.0 mg abbottin (**3**), 2.5 g of hildgardtol A (**4**) 1.5 g of methylhildgardtol A (**5**), 200 mg of 5-*O*-methylobovatin (**6**) and 300 mg of sucrose were obtained.

Crassifolin (**1**), colourless crystals, mp 249–250° $[\alpha]_D$ +4.24 ($CHCl_3$); *c* (3.3). UV λ_{max}, MeOH nm (log ϵ): 248 (4.08), 279 (5.95). IR ν_{max}, $CHCl_3$ cm^{-1}: 3031, 1654, 1215, 901. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75 (6H, *brs*, H-13, H-13″), δ 1.95 (2H, *ddd, J* = 14.1, 12.0, 2.4 Hz, H-3α, H-3″α), δ 2.84 (2H, *dd, H* = 14.4, 12.0, 2.4 Hz, H-3β, H-3″β), δ 2.86 (2H, *dd, J* = 15.3, 9.0 Hz, H-9β, H-9″β), δ 3.16 (2H, *dd, J* = 15.3, 9.6 Hz, H-9α, H-9″α), δ 3.54 (6H, *s*, 2 × OMe), δ 4.86 (2H, *brs*, H-12a, H-12″a), δ 4.90 (2H, *t, J* = 2.4 Hz, H-4α, H-4″α), δ 5.04 (2H, *brs*, H-12b, H-12″b), δ 5.14 (2H, *dd, J* = 9.6, 9.0 Hz, H-10, H-10″), δ 5.37 (2H, *dd, J* = 2.4, 12.0 Hz, H-2β, H-2″β), δ 6.04 (2H, *s*, H-6, H-6″), δ 7.36–7.56 (10H, *m*, phenyl groups); EIMS (70 eV) *m/z* (rel. int.): 658 $[M]^+$ (15), 338 $[C_{21}H_{22}O_4]^+$ (22), 321 $[C_{21}H_{21}O_3]^+$ (100), 320 $[C_{21}H_{20}O_3]^+$ (75), 305 $[C_{20}H_{17}O_3]^+$ (53), 243 (18), 219 (15), 129 (16).

3.2. X-Ray crystal data of crassifolin (*1*)

$C_{42}H_{42}O_7$, MW = 658.8, monoclinic, space group C2, with unit cell dimensions: a = 28.555 (6), b = 4.930 (2), c = 12.460 (2) Å, V = 1754.0 (0) A^3, Z = 2, D_X = 1.247 g ml^{-1}, μ = 0.676 mm^{-1}. Intensity data of colourless needles (0.40 × 0.08 × 0.08 mm) were collected on a Nicolet P_3/F four circle diffractometer using CuKα Ni-filtered radiation (λ = 1.54178 Å). Among them were 3209 unique reflections with $F \geq 4.0\sigma F$ in the range $3° \leq \theta \leq 110°$. The structure was solved by direct methods software provided by the diffractometer manufacturer. The non-hydrogen atoms were anisotropically refined by the full-matrix least-squares method. Hydrogen atoms were located from a different Fourier synthesis. The structure was finally refined to R = 5.18% (wR = 5.64%). Lists of atomic coordinates, thermal parameters, bond lengths and angles, the torsion angles and the calculated and observed structure factors have been deposited at the Cambridge Crystallographic Data Centre, UK.

3.3. Crassichalcone (*2*)

Yellow oil, UV λ_{max} MeOH nm (log ϵ): 348 (4.25), 215 (4.26). IR ν_{max} film cm^{-1}: 3026, 1650, 1604, 1131 and 1110. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.76 (3H, *s*, H-11′), δ 2.94 (1H, *dd, J* = 7.5, 15 Hz, H-7′ax), δ 3.29 (1H, *dd, J* = 9.9, 15 Hz, H-7′eq), δ 3.9 (3H, *s*, OMe), δ 4.92 (1H, *s*, H-10′a), δ 5.07 (1H, *s*, H-10′b), δ 5.28 (1H, *dd, J* = 9.9, 7.5 Hz, H-8′), δ 5.99 (1H, *s*, H-5′), δ 7.38 (3H, *m*, H-3, H-4, H-5), δ 7.57 (2H, *m*, H-2, H-6), δ 7.74 (1H, *d, J* = 15.8 Hz, H-α), δ 7.85 (1H, *d, J* = 15.8 Hz, H-β); EIMS (70 eV) *m/z* (rel. int.): 336 $[M]^+$ (100), 335 [M–H] (37), 321 $[C_{20}H_{17}O_4]$ (20), 259 $[C_{15}H_{15}O_4]$ (66), 233 $[C_{13}H_{13}O_4]$ (8), 131 $[COH_7O]$ (8) 77 $[C_6H_5]$ (6).

3.4. Compound *8*

To a soln of **1** (30 mg) in CH_2Cl_2 (8 ml) was added *p*-toluenesulfonic acid (10 mg) dissolved in Me_2CO (2 ml) which was stirred at room temp. for 10 min. The mixt. was diluted with H_2O and extracted with EtOAc. The residue was purified by CC affording

abbottin **3** (5 mg) and compound **8** (12 mg) as colourless crystals, mp 115–117°: UV λ_{max} MeOH nm (ϵ): 245 (16,741), 307 (7814). IR ν_{max} cm^{-1}: 3018, 1621, 1112, 901. ^1H NMR (200 MHz, $CHCl_3$): δ 1.61 (3H, *brs*, H-13″), δ 1.79 (3H, *brs*, H-13), δ 2.65 (1H, *dd, J* = 15.0, 10.0 Hz, H-9″α), δ 2.95 (1H, *dd, J* = 15.0, 10.0 Hz, H-9α), δ 3.12 (1H, *dd, J* = 15.0, 9.0 Hz, H-9″β), δ 3.30 (1H, *dd, J* = 15.0, 9.0 Hz, H-9β), δ 3.65 (3H, *m*, H-2″, H-4″), δ 3.68 (3H, *s*, OMe), δ 3.73 (3H, *s*, OMe), δ 4.79, 4.98 (2H, *brs*, H-12), δ 4.89, 5.08 (2H, *brs*, H-12″), δ 5.04 (1H, *t, J* = 10.0 Hz, H-10″), δ 5.13 (*m*, H-3″), δ 5.24 (1H, *t, J* = 9.0 Hz, H-10), δ 5.76 (1H, *s*, H-2), δ 5.99 (1H, *s*, H-6), δ 6.06 (1H, H-6″), δ 6.38 (1H, *s*, H-4), δ 6.95 (2H, *m*, H-2‴), δ 7.18 (3H, *m*, H-3‴, H-4‴, H-5‴), δ 7.29 (3H, *m*, H-3′, H-4′, H-5′) and at δ 7.44 (2H, *m*, H-2′, H-6′); EIMS (70 eV) *m/z* (rel. int.): 640 $[M]^+$ (58), 609 $[M–OMe]^+$ (8), 322 $[C_{21}H_{22}O_3]^+$ (23), 321 $[C_{21}H_{21}O_3]^+$ (100), 320 $[C_{21}H_{20}O_3]^+$ (34), 319 $[C_{21}H_{19}O_3]^+$ (44).

Acknowledgements

The authors with to thank R. Gaviño, L. Velasco, M. Torres, and A. Toscano for ^1H NMR, IR, UV, mass spectral and X-ray diffraction analyses.

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