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Absolute Configuration of β-Agarofuran Nucleus of Euojaponine C by CD Exciton Chirality Method

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A new celastraceae alkaloid, euojaponine C has been isolated from the methanol extract of the root bark of *Euonymus japonica*. With the relative stereochemistry of euojaponine C established by NOESY data, the absolute stereochemistry has been determined by circular dichroism (CD) exciton chirality method. The CD of the 2, 5-bis-phenyl benzoate of triacetonide derived from the $LiAlH_4$ hydrolysate, euonyminol shows that the configuration of C-2 and C-5 are both R.

Key words : *Euonymus japonica*, Celastraceae, Alkaloid, Euojaponine C, Absolute configuration, CD exciton chirality, NOESY

INTRODUCTION

In our previous phytochemical study on *Euonymus japonica*, we have isolated twelve alkaloids and identified the structures as new celastraceae family alkaloids which were named as euojaponines (Han *et al.*, 1989; Han *et al.*, 1990a; Han *et al.*, 1990b). Those kinds of alkaloids having β-agarofuran type sesquiterpene nucleus have been extensively studied because of their antifeedant activity against wide range of insects (Gonzalez *et al.*, 1992; Gonzalez *et al.*, 1993). The chemical structures were elucidated by various kinds of modern NMR spectra and chemical modifications (Shizuri *et al.*, 1973; Takaishi *et al.*, 1987; Shirota *et al.*, 1994). In this report, for the determination of absolute stereochemistry of euojaponine C, we applied nuclear Overhauser effects (NOE) in NMR and circular dichroism (CD) exciton chirality method (Harada and Nakanishi, 1983).

The circular dichroic spectroscopy of optically active compounds is a powerful method for studying three-dimensional structures of organic molecules. Namely, the method provides information on the absolute configuration, conformation and reaction mechanism, etc (Lo *et al.*, 1992; Ito *et al.*, 1994; Park *et al.*, 1996). Especially, circular dichroism due to the exciton coupling mechanism provides useful and unambiguous information on absolute configuration and conformation of natural and organic compounds. In the presence of two coupled chromophores in one molecule the excited state splits into two levels and generates Cotton effects of mutually opposite signs. The sign of two split Cotton effects enables us to determine the absolute stereochemistry of the two chromophores in space (exciton chirality). Provided the electric transition dipole moments of the two chromophores constitute a right-handed screwness (positive exciton chirality), the sign of the first Cotton effect is positive and that of the second Cotton effect is negative. When the two dipole moments make a left-handed screwness (negative exciton chirality), the sign of first and second Cotton effects are negative and positive, respectively.

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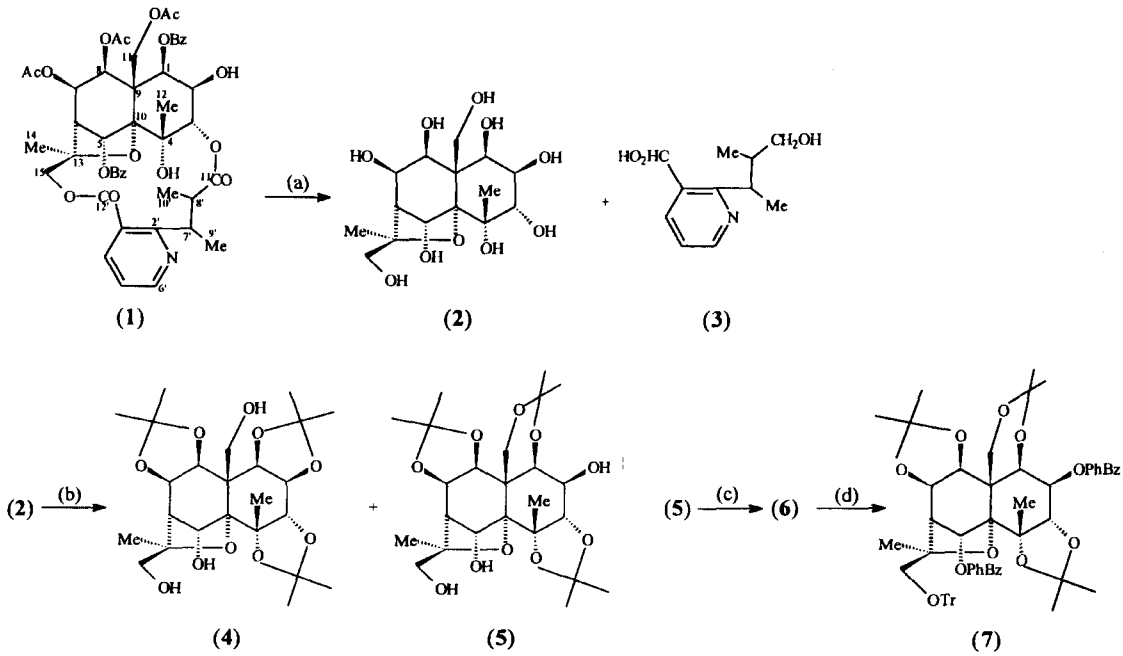
MATERIALS AND METHODS

General experimental procedures

^{1}H-NMR spectra were recorded on Nicolet NT-360 and GE GN-500 spectrometer. Chemical shifts are reported in ppm (δ) relative to $CHCl_3$. Mass spectra were measured on a Hitachi M80-B spectrometer for the secondary ionization mass spectrometry (SIMS). UV/VIS and CD spectra were recorded as CH_3CN solution on a Gilford system 2600 UV/VIS spectrophotometer and Jasco Model J-600A spectropolarimeter, respectively.

Reduction of euojaponine C by $LiAlH_4$

The euojaponine C (**1**) was isolated from the



Scheme 1. Reagents: (a) $LiAlH_4$/THF; (b) 2,2-dimethoxy propane/DMF, camphor sulfonic acid; (c) trityl bromide/pyr; (d) phenyl benzoyl bromide/pyr, DMAP

MeOH extract of the root bark of *Euonymus japonica* as reported previoulsy (Han *et al.*, 1990a). The 85 mg of euojaponine C dissolved in 2 ml of dry THF was added dropwise into the $LiAlH_4$ solution (17 mg in 1 ml of dry THF) with stirring under ice-bath cooling (Yamada *et al.*, 1977). After the further stirring for 14 hrs at room temperature, ice-water was added to decompose the remained $LiAlH_4$, and extracted with EtOAc. The water layer was treated with Amberlite IR-200 (H^+ form) with mild stirring for 20 minutes at room temperature, the solution was filtered and concentrated to afford colorless amorphous residue (euonyminol, **2**, 23 mg). The EtOAc layer was evaporated to give a residue which was purified by preparaive TLC (silica gel) with CH_2Cl_2-MeOH (10:1), giving evoninyl alcohol (**3**, 11 mg) (Scheme 1).

1: mp 188-191°C; IR (KBr) cm^{-1}: 3500, 1740-1720, 1250, 705; UV (λ_{max}, EtOH) nm (log ε): 232 (4.51), 266 (3.62); $[\alpha]_D$: +11.4 (EtOH, c=0.5); EIMS (*m/z*): 887, 844, 828, 782, 766, 722, 700, 107, 105, 43; ^1H NMR ($CDCl_3$, 360 MHz): δ 8.78 (1H, H-6'), 8.14 (1H, H-4'), 7.55 (1H, H-5'), 7.22 (1H, brs, H-5), 6.05 and 3.63 (2H, ABq, *J*=11.4 Hz, H_2-15), 5.78 (1H, d, *J*=3.9 Hz, H-1), 5.56 (1H, dd, *J*=5.9 and 4.2 Hz, H-7), 5.53 and 4.90 (2H, ABq, *J*=13.7 Hz, H2-11), 5.44 (1H, d, *J*=5.9 Hz, H-8), 4.86 (1H, d, *J*=2.5 Hz, H-3), 4.77 (1H, q, *J*=7.1 Hz, H-7'), 4.68 (1H, d, *J*=1.0 Hz, OH at C-4), 4.16 (1H, dd, *J*=3.9 and 2.5 Hz, H-2), 3.12 (1H, brs, OH at C-2), 2.58 (1H, q, *J*=7.1 Hz, H-8'), 2.53 (1H, d, *J*=4.2 Hz, H-6), 1.72 (3H, s, H_3-14), 1.63 (3H, d, *J*=1.0 Hz, H_3-12), 1.47 (3H, d, *J*=7.0 Hz, H_3-9'), 1.17 (3H, d, *J*=7.1 Hz, H-10'), acetyls groups: δ 2.18, 2.34 and 1.42 (3H each, s each), benzoyl protons: δ 8.33, 7.97, 7.59, 7.56, 7.50, 7.41

3: ^1H NMR ($CDCl_3$, 360 MHz): δ 8.51 (1H, dd, *J*= 4.8 and 1.7 Hz, H-6'), 7.67 (1H, dd, *J*=7.7 and 1.7 Hz, H-4'), 7.14 (1H, dd, *J*=7.7 and 4.8 Hz, H-5'), 4.87 and 4.50 (2H, ABq, *J*=12.3 Hz, H_2-12'), 3.36 (2H, d, *J*=3.4 Hz, H_2-11'), 3.27 (1H, dq, *J*=8.9 and 6.9 Hz, H-7'), 2.18 (1H, m, H-8'), 1.23 (3H, d, *J*=6.9 Hz, H_3-9'), 1.02 (3H, d, *J*=7.0 Hz, H_3-10')

Triacetonides of euonyminol (4 and 5)

The solution of euonyminol (20 mg) in dry DMF (2 ml) was added 2,2-dimethoxy propane (1 ml) and D, L-camphor sulfonic acid (30 mg) (Shizuri *et al.*, 1973). The mixture was stirred at 45°C for 5 hr and diluted with saturated $NaHCO_3$ aquous solution and then evaporated, giving a residue, which showed two spots on TLC. Separation of the residue by silica gel flash chromatography with CH_2Cl_2-MeOH (70:1) afforded triacetonide I (**4**, 7.8 mg, amorphous powder, upper spot) and triacetonide II (**5**, 4.6 mg, amorphous powder, lower spot) (Scheme 1). **4**: LSIMS: $[M+H]^+$ 487, $[M+Na]^+$ 509; ^1H NMR ($CDCl_3$, 360 MHz): δ 4.73 (1H, dd, *J*=7.6 and 2.1 Hz, H-7), 4.71 (1H, brs, H-5), 4.48 (1H, d, *J*=7.6 Hz, H-8), 4.34 (1H, d, *J*=4.0 Hz, H-1), 4.34 (1H, d, *J*=1.6 Hz, H-3), 4.26 (1H, dd, *J*=4.0 and 1.6 Hz, H-2), 4.09 and 3.22 (2H, ABq, *J*= 13.8 Hz, H_2-11), 3.64 and 3.55 (2H, ABq, *J*=12.2 Hz, H_2-15), 2.52 (1H, d, *J*=2.1 Hz, H-6), methyl groups:

δ 1.94, 1.57, 1.52, 1.50, 1.45, 1.38, 1.31 and 1.28 (3H each, s each); **5**: LSIMS: $[M+H]^+$ 487, $[M+Na]^+$ 509; ^1H NMR ($CDCl_3$, 360 MHz): δ 4.61 (1H, dd, *J*= 5.5 and 2.9 Hz, H-7), 4.36 (1H, ddd, *J*=10.8, 6.5 and 2.6 Hz, H-2), 4.26 (1H, brs, H-5), 4.10 (1H, d, *J*=5.5 Hz, H-8), 4.08 (1H, d, *J*=6.5 Hz, H-1), 4.02 and 3.85 (2H, ABq, *J*=12.0 Hz, H_2-11), 3.92 (1H, d, *J*=2.6 Hz, H-3), 3.73 and 3.53 (2H, ABq, *J*=12.8 Hz, H_2-11), 2.68 (1H, d, *J*=2.9 Hz, H-6), 2.31 (1H, d, *J*=10.8 Hz, OH at C-2), methyl groups : δ 1.95, 1.60, 1.56, 1.49, 1.46, 1.43, 1.35 and 1.32 (3H each, s each)

Preparation of chromophoric derivative

To a solution of **5** (1 mg) in pyridine (0.5 ml) trityl bromide (1.5 mg) was added and stirred at 50°C for 8 hr. The mixture was dried up *in vacuo* and purified by silica gel flash chromatography with CH_2Cl_2-MeOH (50:1) to afford **6**. A solution of **6** (1 mg) in pyridine (1 ml) was added phenyl benzoyl bromide (0.8 mg) and 4-N,N-dimethylamino pyridine (DMAP, 5 mg) and stirred at 80°C for 12 hr. The mixture was dried *in vacuo* and purified by silica gel flash chromatography with CH_2Cl_2-MeOH (100:1) to afford **7** (0.47 mg) (Scheme 1). **6**: LSIMS: $[M+H]^+$ 729; UV (λ_{max}, CH_3CN) nm (log ε): 199 (4.48); **7**: LSIMS: $[M+H]^+$ 1061; UV (λ_{max}, CH_3CN) nm (log ε): 204.0 (5.08), 273.0 (4.69); CD λ_{ext}, nm (Δε): 272.0 (-2.05), 223.8 (+4.05); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.8-7.2 (33H, aromatic protons), 7.24 (1H, brs, H-5), 4.64 (1H, dd, *J*=5.6 and 3.0 Hz, H-7), 5.54 (1H, dd, *J*=7.1 and 2.6 Hz, H-2), 4.10 (1H, d, *J*=7.1 Hz, H-1), 4.10 (1H, d, *J*=5.6 Hz, H-8), 3.98 (1H, d, *J*=2.6 Hz, H-3), 3.94 and 3.80 (2H, ABq, *J*=12.7 Hz, H_2-11), 3.30 (1H, d, *J*=2.9 Hz, H-6), 2.94 and 2.76 (2H each, ABq, *J*=12.3 Hz, H_2-15), methyl groups: δ 1.84, 1.60×3, 1.48, 1.32, 1.30 and 1.04 (3H each, s each)

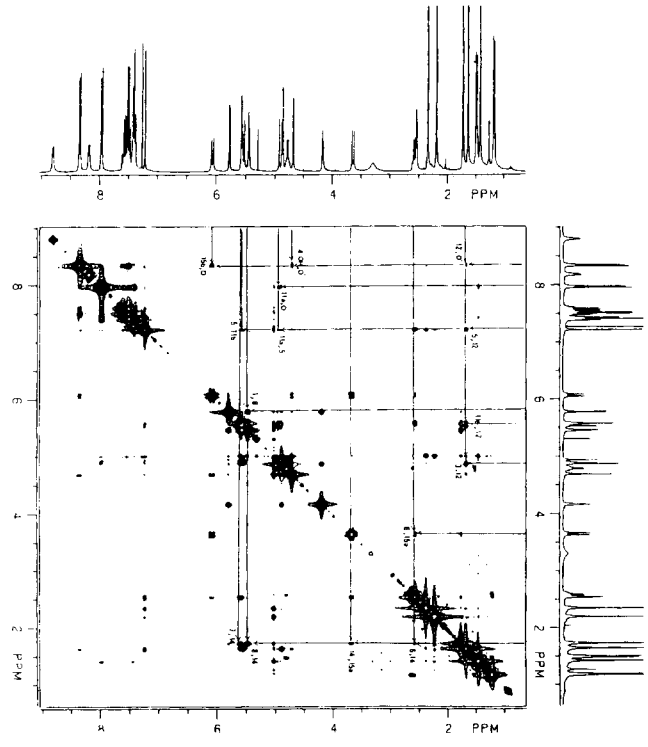


Fig. 1. NOESY spectra of euojaponine C ($CDCl_3$, 360 MHz).

RESULTS AND DISCUSSION

The chemical structure of euojaponine C was confirmed by spectroscopic data as reported elsewhere and the NMR spectral assignments were completed by using two dimensional NMR study (Han *et al.*, 1990a). The relative stereochemistry of **1** was elucidated on the basis of NOESY correlations (Fig. 1) and ^1H-^1H coupling constants. The formation of cyclodiester ring between C-15 and C-3, and the NOESY specta showing cross peaks of H_2-11/H-5, H-5/H_3-12, $H_{b\text{-}11}$/H_3-12 indicate a chair conformation for both A and B rings and a trans-junction between rings A and B. Therefore all the protons of H_3-12, H-5 and H_2-11 are located on the same side of the rings. From the very weak ^1H-^1H coupling between H-5 (brs) and H-6 and structural model study, H-6 revealed as β-conformation. And ring C is formed by the 6, 10 diaxial bond linkage. The ester bond at C-3 was assigned as being α-orientation from the NOESY correlation of H-3/H_3-12 and the formation of acetonide between the hydroxy groups at C-3 and C-4 of euonyminol. NOESY correlations of H_3-14/H-7, H_3-14/H-8, and H-8/H-1 implicated that H_3-14, H-7, H-8 and H-1 are all α-oriented, while the ^1H-^1H coupling constant between H-1 and H-2 (*J*=3.9 Hz) revealed the α-orientation of H-2.

In order to determine the absolute streochemistry of the β-agarofuran nucleus of euojaponine C by CD exciton chirality method (Harada and Nakanishi, 1983), euojaponine C was chemically modified for the in-

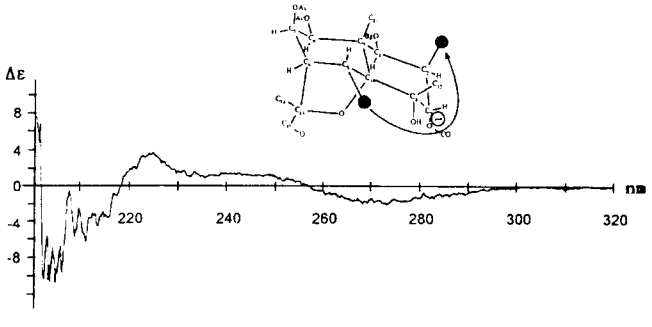


Fig. 2. CD spectra of 2,5-bis-phenyl benzoyl triacetonide II of euojaponine C (**7**) in CH_3CN. Inserted structure is sesquiterpene nucleus of euojaponine C and hatched circles is the position of phenyl benzoyl chromophores introduced into C-2 and C-5.

troduction of two chromophores at appropriate positions. Scince euojaponine C possesses many acyl groups and diester bonds, it was hydrolysed by $LiAlH_4$ to yield euonyminol (**2**) and evoninyl alcohol (**3**). The β-agarofuran type sesquiterpene polyol was converted to two triacetonide compounds (**4** and **5**). Triacetonide II (**5**) has two free secondary hydroxy groups at C-2 and C-5 suitable for the introduction of chromophores with a dihedral angle of 90°C, which is the best angle for the CD splitting. After blocking the primary hydroxy group at C-15 by tritylation, 2,5-bis-phenyl benzoyl derivative (**7**) of **6** was prepared. The UV spectra measured in CH_3CN indicated that the two secondary alcohol was converted into phenyl benzoate. As shown in Fig. 2 complete split CD spectra were observed, which originated from the interaction between two chromophores. They are bathochromic shifted a little because of the presence of trityl group at C-15, but the effect of trityl group on chromophoric coupling and the sign of exciton chirality was negligible. From the split CD band with extreme at 272.0 nm (-2.05) and 223.8 nm (+4.05), with A value of -6.10, it was concluded that the chromophoric derivative exhibit negative exciton chirality. These data indicate that the two chromophores constitute a counter-clockwise exciton chirality. With the relative stereochemistry of euojaponine C established by NOESY data, the absolute configurations at C-2 and C-5 determined as 2R and 5R.

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REFERENCES CITED

Gonzalez, A. G., Jimenez, I. A., Ravelo, A. G., Belles, X. and Piulachs, M. D., Antifeedant activity of dihydro-β-agarofuran sesquiterpenes from celastraceae against *Spodoptera littoralis, Biochem. System. Ecol.* 20, 311-315 (1992).

Gonzalez, A. G., Jimenez, I. A., Ravelo, A. G., Sazatornil, J. G. and Bazzocchi, I. L., New sequiterpenes with antifeedant activity from *Maytenus canariensis* (Celastraceae), *Tetrahedron*, 49, 697-702 (1993).

Han, B. H., Park, M. K., Ryu, J. H., Park, J. H., Naoki, H., Han, Y. N. and Huh, B. H., Polyester type sesquiterpene alkaloids from *Euonymus japonica* (structures of euojaponine-D, -F, -G, -J and -K), *Arch. Pharm. Res.*, 12, 306-309 (1989).

Han, B. H., Park, M. K., Ryu, J. H., Park, J. H. and Naoki, H., Sesquiterpene alkaloids from *Euonymus japonica, Phytochem.*, 29, 2303-2307 (1990a).

Han, B. H., Ryu, J. H., Han, Y. N., Park, M. K., Park, J. H. and Naoki, H., New sesquiterpene alkaloids from *Euonymus japonica*: (structures of euojaponine D, F, J and K), *J. Nat. Prod.*, 53, 909-914 (1990b).

Harada, N. and Nakanishi, K., *Circular dichroism spectroscopy-Exciton coupling in organic stereochemistry*; University Science Books, Mill Valley, 1983.

Katsuta, Y., Ito, M., Yoshihara, K., Nakanishi, K., Kikkawa, T. and Fujiwara, T., Synthesis of (+)-(4S)- and (-)-(4R)-(11Z)-4-Hydroxyretinals and determination of the absolute stereochemistry of a visual pigment chromophore in the firefly squid, *Watasenia scintillans, J. Org. Chem.*, 59, 6917-6921 (1994).

Lo, L.-C., Berova, N., Nakanishi, K., Schlingmann, G., Carter, G.T. and Borders, D. B., Determination of the absolute stereochemistry of nemadectins α2, and α, *J. Am. Chem. Soc.*, 114, 7371-7374 (1992).

Park, M. H., Suh, D.-Y. and Han, B. H., Absolute configuration of a cyclopeptide alkaloid, sajoinine-G_1, from *Zizyphus vulgaris var. spinosus, Phytochem.*, 43, 701-704 (1996).

Shirota, O., Otsuka, A., Morita, H., Takeya, K. and Itokawa, H., Sesquiterpene pyridine alkaloids from *Maytenus chuchuhuasca, Hetrocycles*, 38, 2219-2229 (1994).

Shizuri, Y., Sugiura, A. K., Yamada, K. and Hirata, Y., The structures of evonine and neoevonine alkaloids *obtained* from *Euonymus sieboldiana* Blume, *Tetrahedron*, 29, 1773-1794 (1973).

Shizuri, Y., Wada, H., Yamada, K. and Hirata, Y., *Tetrahedron*, 29, 1795-1800 (1973).

Takaishi, Y., Ujita, K., Noguchi, H. and Kikuchi, T., Structures of Triptofordinine A-1 and A-2 determined by 2-D NMR spectroscopy, *Chem. Pharm. Bull.* 35, 3534-3537 (1987).

Yamada, K., Sugiura, K., Shizuri, Y., Wada., H. and Hirata, Y., Isolation and structure of euonymine and neoeuonymine, alkaloids from *Euonymus sieboldiana* Blume, *Tetrahedron*, 33, 1725-1728 (1977).