Synthesis of [60]Fullerene-Podophyllotoxin Derivative†

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The [60]fullerene-podophyllotoxin derivative (3) was obtained by the phosphine promoted [2 + 3] cycloaddition reaction of podophyllotoxin buta-2,3-dienoate (2) and [60]fullerene. The structures of starting material (2) and product (3) were confirmed by UV-vis, IR, NMR and MS spectroscopies.

Keywords [60]fullerene, podophyllotoxin, [2 + 3]cycloaddition, natural product

Introduction

Podophyllotoxin (**1**) is a well known natural product endowed with potent antineoplastic activity.[1] The synthesis and preparation of different types of derivatives of this molecule have been attracting much interest of both organic chemists and medicinal chemists.[2] In this paper, the synthesis of a novel and interesting derivative of **1** is reported, in which **1** is introduced to the [60]fullerene (C_{60}) cage.

The method we adopted is the phosphine catalyzed [2 + 3]cycloadditon of buta-2,3-dienoate with C_{60}. This reaction was previously studied.[3] However, little attention has been directed toward expanding this type of reaction toward more complex adducts. Aiming to explore the reaction further and seek an easy method to constructing C_{60} derivative containing podophyllotoxin moiety for the purpose of evaluating the potential biological properties of this novel molecule, we conducted this investigation.

Results and discussion

Previous studies[4] of the structure-activity relationship showed that the *trans*-fused γ-lactone moiety was an important factor for displaying significant antineoplastic activity, the *cis* analogue being less potent. If the γ-lactone ring and the C-2 configuration are kept intact, the C-4 is one of the effective modification sites. Cautions should be taken when **1** is treated with basic reagents, since the C-2/C-3 *trans* structure would likely be converted to the more thermodynamically stable C-2/C-3 *cis* structure. As a result, the 1,2-*cis* and 2,3-*trans* structures that are required for the anti-tumor activities would disappear.

The structure of the [60]fullerene podophyllotoxin derivative (**3**) synthesized in this report is shown in Scheme 1. The synthesis is straightforward, consisting of only two steps. To our surprise, the treatment of **1** with tetrolyl chloride in dichloromethane at room temperature in the presence of triethylamine resulted in the formation of the buta-2,3-dienoate (**2**) instead of the expected but-2-ynoate. The IR (KBr) spectrum of **2** indicated the presence of a cumulated double-bond system (absorbing at 1969 cm^{-1}, CH = C = CH_2) and the absence of an alkyne group (no C≡C band was observed in the region of 2260—2100 cm^{-1} for alkynes).[5] The 300 MHz proton NMR spectrum ($CDCl_3$) showed a one-proton triplet (δ 5.72, J = 6.5 Hz, CH = C = CH_2) and two almost-overlapped doublets(δ 5.281 and 5.278, J = 6.6, 6.4 Hz,

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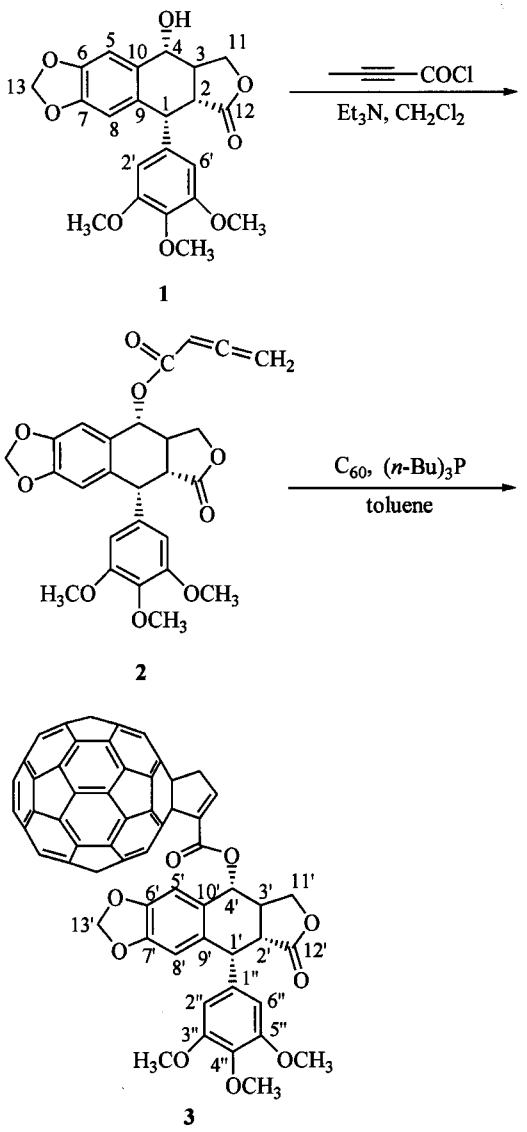
Scheme 1



$CH=C=CH_2$), consistent with the structure of $CH=C=CH_2$. However, no methyl signal was observed in the region of δ 1.6—2.0 for $C\equiv C-CH_3$. It should be indicated that it is the first time to report the triethylamine-catalyzed isomerization of an ynoate to a 2,3-dienoate, though a 2,3-dienoate has been hypothesized to be an intermediate in the phosphine-catalyzed isomerization of ynoates to 2,4-dienoates.[6]

Fortunately, the mild esterification conditions should not give rise to the alteration of the γ-lactone ring, which was confirmed by the IR and ^1H NMR data. The IR spectrum gave a very strong absorption band at 1779 cm^{-1} characteristic of the γ-lactone ring. In the ^1H NMR spectrum, H-1 and H-4 resonated at 4.61 (d, $J_{1,2}=3.7$ Hz) and 5.95 (d, $J_{3,4}=7.4$ Hz), respectively. Clearly, the $J_{1,2}$ and $J_{3,4}$ values of **2** are consistent with those of **1** and podophyllotoxin acetate (Table 1),[7] indicating the configurations of C-2 and C-4 remain.

Table 1 Comparison of $J_{1,2}$, $J_{2,3}$ and $J_{3,4}$ of the analogues

	$J_{1,2}$ (Hz)	$J_{2,3}$ (Hz)	$J_{3,4}$ (Hz)
1	~3		8
Podophyllotoxin acetate	3.5		8.0
2	3.7	14.4	7.4
3	4.2	14.4	8.4
Epipodophyllotoxin	5.0	14	3.5
Epipodophyllotoxin acetate	4.75	14	3.5
Picropodophyllin	6.75	9.0	9

It has been reported that buta-2,3-dienoates are more reactive than but-2-ynoates towards C_{60}, and for the former, forcing conditions are avoidable.[3b] Indeed, although it had the bulky moiety of **1**, the attempted reaction of buta-2,3-dienoate (**2**) with C_{60} in the presence of tributylphosphine underwent smoothly under mild conditions. The reaction was carried out in toluene at room temperature for only 40 min, producing the desirable product **3** in good yield (70%, based on consumed C_{60}), which possesses good solubility in common organic solvents such as carbon disulfide, toluene, dichloromethane and chloroform. Separation of **3** was readily accomplished by flash chromatography on silica gel using CH_2Cl_2-EtOAc as eluent. It was found that a 1:1 molar ratio of **2** and C_{60} was optimal, as an excess of **2** would result in a lower yield of **3** due to marked increase in the proportion of products arising from multiple additions to the cage. A 5:1 molar ratio of **2** to C_{60} caused complete consumption of C_{60} within 10 min, but almost no monoadduct **3** except multiple adducts was observed.

The structure of **3** was determined on the basis of MS, IR and NMR spectral data. The FD-MS spectrum gave the base molecular ion peak at m/z 1200. The FT-IR spectrum (KBr pellet) displayed absorption bands at 1779 (γ-lactone C=O), 1713 (α,β-unsaturated ester C=O), along with a very strong band at 526 cm^{-1} characteristic of the fullerene cage. In the ^1H NMR (600 MHz, $CDCl_3$) spectrum, the olefinic proton and its adjacent methylene protons of the C_{60}-fused five-member ring appeared at 7.92 (t, $J=2.4$ Hz, 1H) and 4.65 (d,

J = 2.4 Hz, 2H), respectively. In the podophyllotoxin moiety, H-1′, H-2′, H-3′ and H-4′ protons appeared at 4.64 (d, $J_{1',2'}$ = 4.2 Hz, 1H), 2.98 (dd, $J_{1',2'}$ = 4.2, $J_{2',3'}$ = 14.4 Hz, 1H), 3.01—3.08 (m, 1H) and 6.13 (d, $J_{3',4'}$ = 8.4 Hz, 1H). Apparently, the $J_{1',2'}$, $J_{2',3'}$ and $J_{3',4'}$ values of **3** are in accordance with those of **1** and **2** (Table 1), also proving that the configurations of C-2′ and C-4′ unchange. In the ^{13}C NMR spectrum, the characteristic carbonyl signals for the γ-lactone and α,β-unsaturatee ester appeared at δ 173.53 and δ 163.87, respectively.

From figure of the sp^2 carbon region of δ 105—160, apart from the carbon signals pertaining to the two benzene rings and the two olefinic carbons in the C_{60}-fused five-member ring, there are a total of 30 sp^2 carbon signals corresponding to the fullerene cage (see the experimental part). In the sp^3 carbon region, in addition to the C-13′ that was expected to appear at δ 101.72, a total of 10 sp^3 signals were observed in the range of δ 0—90, of which 8 signals were shown to be the protonated sp^3 carbons of the podophyllotoxin moiety by the HMQC (heteronuclear multiple-quantum coherence) experiment. Undoubtedly, the remaining 2 quaternary sp^3 carbon signals at δ 76.56 and δ 69.68 are the characteristic fullerene sp^3 carbons. The 30 fullerene sp^2 carbon signals combined with the two fullerene quaternary sp^3 carbon signals confirm a C_s symmetry of the C_{60} cage, clearly indicating a 6,6-closed addition pattern on the fullerene cage.

Thus, the phosphine catalyzed [3 + 2] cycloadditon of buta-2,3-dienoate with [60]fullerene has been shown to be an effective method for the synthesis of the fullerene-podophyllotoxin derivative. In the derivative obtained, the γ-lactone ring, which is critical for the biological properties, and its stereo structure were kept intact. This novel derivative is interesting structurally, and for its potential biological applications.

Experimental

UV-vis spectra were obtained on a Shimadzu UV-240 spectrometer. IR spectra were recorded on a Nicolet FT IR-5DX spectrometer. FD-MS spectra were recorded on a Finnigan MAT 90 mass spectrometer and TOF-SIMS were recorded on a TOF-2000MI high resolution mass spectrometer. NMR data were obtained from Brucker 300 and 600 spectrometers and recorded in $CDCl_3$ solution. Chemical shift values (δ) are reported and in relation to TMS (δ 0.00) and $CDCl_3$ (δ 77.00) for ^1H NMR and ^{13}C NMR, respectively. Toluene was distilled over sodium. Methylene chloride was dried over $CaCl_2$ and distilled. The yield of product **3** is based on consumed C_{60}. The recovered C_{60} was washed with ether and methanol, respectively, in order to correctly calculate the yield of product **3**.

Tetrolic acid was prepared according to the literature procedure[8] (white crystal, m.p. 77 ℃, lit. 77 ℃). Tetrolyl chloride was made from tetrolic acid by refluxing in oxalyl chloride for 3 h based on the reported method.[9a] The excess oxalyl chloride should be completely removed by distillation under reduced pressure to obtain pure tetrolyl chloride, colorless liquid, IR (neat) ν: 2226 (C≡C), 1754 (C=O), 1163, 837 cm^{-1}, consistent with literature data;[9b] ^1H NMR (60 MHz, CCl_4) δ: 2.03 (s, CH_3). Tributylphosphine was freshly prepared according to the literature method.[10]

Synthesis of compound **2**

To an ice-cold solution of **1** (0.94 g, 2.17 mmol) and triethylamine (0.31 g, 3.03 mmol) in methylene chloride (20 mL) was added dropwise a solution of tetrolyl chloride (0.34 g, 3.26 mmol) in methylene chloride (10 mL). The resulting mixture was stirred at room temperature overnight. Cold water (20 mL) was added to quench the excess reagent. The aqueous mixture was extracted with methylene chloride twice. The combined organic phase was dried over anhydrous Na_2SO_4. After removal of the solvent, the crude product was subjected to flash column chromatography on silica gel using CH_2Cl_2-EtOAc (30∶1, V∶V) as eluent to afford **2** (0.49 g, yield 45%).

2 ^1H NMR (300 MHz, $CDCl_3$) δ: 6.79 (s, 1H, H-5), 6.54 (s, 1H, H-8), 6.39 (s, 2H, H-2′ + H-6′), 5.99 (AB, J = 0.9 Hz, 2H, H-13), 5.95(d, J = 7.4 Hz, 1H, H-4), 5.72 (t, J = 6.5 Hz, 1H, allenic H), 5.281 (d, J = 6.6 Hz, 1H, allenic H), 5.278 (d, J = 6.4 Hz, 1H, allenic H), 4.61 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.41 (dd, J = 9.3, 6.4 Hz, 1H, H-11), 4.21(dd, J = 9.3, 9.5 Hz, 1H, H-11), 3.81 (s, 3H, OCH_3), 3.78 (s, 6H, 2 OCH_3), 2.92 (dd, $J_{1',2'}$ = 3.7 Hz, $J_{2',3'}$ = 14.4 Hz, 1H, H-2), 2.89—2.93 (m, 1H, H-3); IR (KBr) ν: 1969 (CH=C=CH_2), 1779 (lactone C=O), 1713 (ester C=O) cm^{-1}; EI-MS m/z (%): 480 (M^+, base peak), 397 (29.17), 351 (8.34), 313 (10.93), 282 (9.97), 229

(10.28), 185 (18.64), 168 (24.12); HRMS calad for $C_{20}H_{24}O_9$ 480.1420, found 480.1374.

Synthesis of compound **3**

A mixture of C_{60} (37.0 mg, 0.051 mmol) and tributylphosphine (42 mg, 0.208 mmol) in toluene (30 mL) was stirred under N_2 at room temperature until a complete dissolution of C_{60}. To this solution was added **2** (23 mg, 0.048 mmol, 0.94 equiv.). The resulting mixture was stirred at room temperature for 40 min. The color of the solution changed from purple to dark brown. Without concentration, the reaction solution was directly subjected to flash chromatography on silica gel first using toluene as eluent to furnish unreacted C_{60} (10.6 mg), then using CH_2Cl_2-EtOAc (50:1, *V*:*V*) as eluent to afford **3** (30.9 mg, yield 70%, based on consumed C_{60}).

3 UV-vis(CH_2Cl_2) λ_{max}: 229, 255, 322 (sh), 430 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.92 (t, J = 2.4 Hz, 1H, the olefinic proton of C_{60}-fused five-member ring), 6.96 (s, 1H, H-5′), 6.59 (s, 1H, H-8′), 6.46 (s, 2H, H-2″ + H-6″), 6.13 (d, J = 8.4 Hz, 1H, H-4′), 6.01 (s, 2H, H-13′), 4.66 (d, J = 2.4 Hz, 2H, CH_2 of C_{60}-fused five-member ring), 4.65 (d, J = 4.2 Hz, 1H, H-1′), 4.47 (dd, J = 9.0, 6.6 Hz, 1H, H-11′), 4.31 (dd, J = 9.0, 10.6 Hz, 1H, H-11′), 3.83(s, 3H, OCH_3), 3.81 (s, 6H, 2 × OCH_3), 3.01—3.08 (m, 1H, H-3′), 2.99 (dd, J = 4.2, 14.4 Hz, 1H, H-2′); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ: 38.87 (C-3′), 43.82 (CH_2 of C_{60}-fused five-member ring), 45.75 (C-2′), 47.69 (C-1′), 56.49 (2C, 2 × OCH_3), 60.81 (OCH_3), 69.68 (sp^3-C of C_{60}), 71.49 (C-11′), 75.02 (C-4′), 76.56 (sp^3-C of C_{60}), 101.72 (C-13′), 107.26 (C-5′), 108.69 (C-2″ + C-6″), 110.00 (C-8′), 128.00 (1C), 132.63 (1C), 134.57 (2C), 134.85 (1C), 135.89 (1C), 135.97 (2C), 137.67 (1C), 139.33 (1C), 139.40 (1C), 140.37 (2C), 141.60 (2C), 141.95 (1C), 142.07 (2C), 142.26 (2C), 142.41 (2C), 142.74 (2C), 142.81 (2C), 143.17 (2C), 144.41 (2C), 144.54 (2C), 145.00 (2C), 145.21 (2C), 145.34 (2C), 145.45 (2C), 145.63 (2C), 145.83 (= CH – of C_{60}-fused five-member ring), 146.07 (3C), 146.24 (2C), 146.35 (2C), 146.41 (2C), 147.35 (2C), 147.42 (2C), 147.67 (2C), 148.02 (2C), 148.36 (2C), 149.08 (1C), 149.28 (1C), 152.79 (2C), 155.87 (2C), 163.87 (ester C=O), 173.53 (lactone C=O). The HMQC spectrum confirmed the assignment of the carbon signals. FT-IR (KBr) ν: 1779 (lactone C=O), 1713 (ester C=O), 1637, 1481, 1230, 1124, 1083, 997, 526 cm^{-1}; FD-MS *m*/*z* (%): 1200 (M^+, 100), 804 (97).

References

1 (a) Jardine, I. In *Anticancer Agents based on Natural Products Models*, *Chapter 9*, Eds.: Cassady, J. M.; Douros, J. D., Academic Press, New York, **1980**, pp. 319—351.
(b) Weiss, S. G.; Tin-Wa, M.; Perdue, R. E.; Farnswoth, N. R. *J. Pharm. Sci.* **1975**, *64*, 95.

2 (a) Damayanthi, Y.; Lown, J. W. *Curr. Med. Chem.* **1998**, *5*, 205.
(b) Ward, R. S. *Nat. Prod. Rep.* **1999**, *16*, 75.
(c) Botta, B.; Delle, M. G.; Misiti, A.; Vitali, A.; Zappia, G. *Curr. Med. Chem.* **2001**, *8*, 1363.
(d) Ward, R. S. *Synlett* **1992**, 719.
(e) Ward, R. S. *Tetrahedron* **1990**, *46*, 5029.
(f) Ward, R. S. *Chem. Soc. Rev.* **1982**, *11*, 75.

3 (a) Shu, L.-H.; Sun, W.-Q.; Zhang, D.-W.; Wu, S.-H.; Wu, H.-M.; Xu, J.-F.; Lao, X.-F. *Chem. Commun.* **1997**, 79.
(b) O'Donovan, B. F.; Hitchcock, P. B.; Meidine, M. F.; Kroto, H. W.; Taylor, R.; Walton, D. R. M. *Chem. Commun.* **1997**, 81.
(c) Zhang, C.; Lu, X.-Y. *J. Org. Chem.* **1995**, *60*, 2906.

4 (a) Sackett, D. L. *Pharmacol. Ther.* **1993**, *59*, 163.
(b) Issell, B. F. *Etoposide* (*Vp-16-213*), *Current Status and New Developments*, Academic Press, New York, **1984**, pp. 21—25.

5 Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 6th ed., John Wiley & Sons, Inc., New York, **1998**, pp. 85—86.

6 (a) Trost, B. M.; Kazmaier, U. *J. Am. Chem. Soc.* **1992**, *114*, 7933.
(b) Guo, C.; Lu, X.-Y. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1921.

7 Ayres, D. C.; Harris, J. A.; Jenkins, P. N.; Phillips, L. *J. Chem. Soc., Perkin Trans. 1*, **1972**, 1343.

8 Simmross, F.-M.; Weyerstahl, P. *Synthesis* **1981**, 72.

9 (a) Ali, S. A.; Powell, J. W.; Whalley, W. B. *J. Chem. Soc. Perkin Trans. 1* **1973**, 173.
(b) Augdahl, E.; Kloster-Jensen, E.; Rogstad, A. *Spectrochim. Acta* **1974**, *30A*, 399.

10 Kauffman, G. B.; Teter, L. A. *Inorg. Synth.* **1960**, *6*, 87.

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