Electrochemical Oxidation of Anti-tumor Agent, Etoposide

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As part of a search for new potent derivatives, electrochemical oxidation of etoposide (1) was carried out under controlled potential (500 mV) to yield 1,2-dehydroetoposide (4), 4′-*O*-demethyl-1,2,3,4-tetradehydro-4-dehydroxy-podophyllotoxin (5) and 1,2,3,4-tetradehydroetoposide (6). They showed no cytotoxicity against B16-melanoma.

Keywords etoposide; anti-tumor agent; dehydroetoposide, electrochemical oxidation; controlled potential

Etoposide (**1**) is a semi-synthetic anti-tumor agent. Recently, Holthuis *et al.*, have reported[1] that two-electron electrochemical oxidation (850 mV, 0.2 M KH_2PO_4, pH 4.6) of **1** afforded the orthoquinone derivative (**2**) (Fig. 1). The authors speculated that **2** was formed *via* the stable radical intermediate (**3a** or **3b**, Chart 1) generated in the first voltammetric oxidation step, by the second electron-transfer and release of methanol. In order to investigate further the behavior of **3b**, in the expectation of finding new derivatives having more potent anti-tumor activity, **1** was electrolyzed at lower potential. In this paper we wish to report the lower-potential electrolytic oxidation of etoposide to yield two new derivatives together with the reported one.

A solution of etoposide (**1**, 0.1 mmol) in methanol (20 ml) containing sodium perchlorate (0.02 M) as a supporting electrolyte was electrolyzed at a constant potential (500 mV) using a potentio-galvanostat. HPLC of the reaction mixture showed three components **4**, **5** and **6** in a ratio of 20 : 7 : 47. Careful separation of the mixture on a reversed phase silica gel column afforded **4**, **5** and **6** in 9, 1 and 26% yields, respectively. Compound **4** was a 1,2-dehydro derivative of **1**, which showed peaks at *m/z* 587 $(M+H)^+$ and 381 $(M-(\text{glycoside}-OH)+H)^+$ in the mass spectrum (FAB) and no peak due to vicinal 1- and 2-protons of **1** in the ^{1}H-NMR spectrum, together with an absorption at longer wavelength than that of **1** in the UV spectrum. The aromatized des-sugar compound **5** showed a peak at *m/z* 381 $(M+H)^+$ in the mass spectrum. The absorptions of the sugar moiety and 1-, 2-, 3- and 4-protons were not observed but a new olefinic 4-proton signal was seen at δ 7.69 ppm in the ^{1}H-NMR ($CDCl_3$) spectrum. Compound **6** showed peaks of *m/z* 585 $(M+H)^+$ and 397 $(M-\text{glycocyl}+H)^+$ in the mass spectrum (MS), and there was no 4-proton signal in the ^{1}H-NMR spectrum. Furthermore, **6** was identical

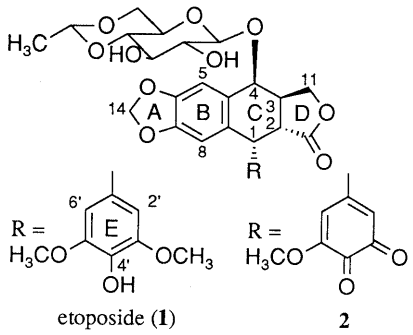

Fig. 1

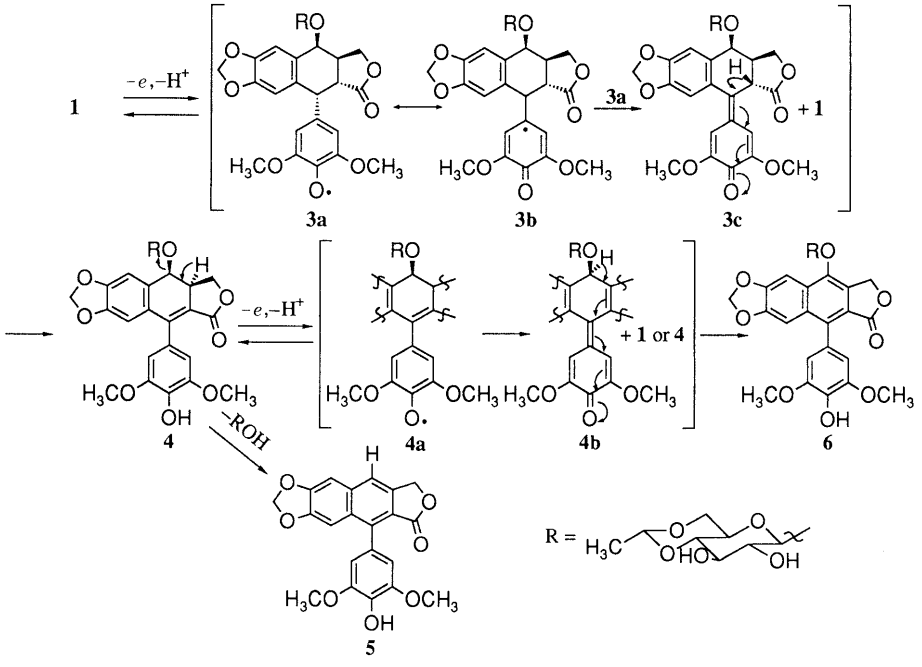

Chart 1

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TABLE I. Effects of Electrolytes on the Ratio of the Electrolysis Products

Entry No.	Potential (mV)	Supporting electrolyte		Ratio of products (%)			
		Kind	Conc. (M)	1	4	5	6
1	500	$NaClO_4$	0.02	22	20	7	47
2	500	Et_4NOTs	0.02	13	15	43	18
3	500	Et_4NOH	0.02	20	41	15	23
4	500	$NaClO_4$	0.2	35	18	6	28
5	250	Et_4NOH	0.02	Trace	Trace	47	11

with the reported compound, based on a comparison of the ^{1}H-NMR spectrum.[2]

A plausible mechanism for these reactions is shown in Chart 1. After one-electron transfer and deprotonation,[3] a radical **3a** is generated, and it resonates with **3b**. Radicals **3a** and **3b** disproportionate to generate **3c**[4] and **1**. Migration of the 2-proton of **3c** to the 4′-oxygen atom and subsequent sugar elimination afford **4** and **5**, respectively. One-electron transfer and deprotonation of **4** give **4a**, which yields **4b**, probably by a similar process to that of formation of **3c** from **1**, and **4b** in turn gives **6**.

In order to investigate the effect of supporting electrolytes in this oxidation, the electrolysis of **1** was carried out in the presence of tetraethylammonium tosylate and tetraethylammonium hydroxide besides sodium perchlorate and the ratios of the products were determined by HPLC. Table I summarizes the ratios of these products obtained under various electrolysis conditions. The ratio of products depended on the kind of supporting electrolyte. When sodium perchlorate was used (entry 1), the major product was **6** and the ratio of **4**:**5**:**6** was 20:7:47. However, in experiments using tetraethylammonium tosylate (entry 2), the ratio was changed and the major product was **5**. Electrolysis with tetraethylammonium hydroxide (entry 3) gave **4** as a major product. In the case of tetraethylammonium hydroxide, the ratio did not depend significantly on the concentration of the electrolyte (entry 4), but **5** was generated as a major product when the potential was dropped to 250 mV from 500 mV (entry 5).

None of the products (**4**, **5** and **6**) obtained in this study showed *in vitro* cytotoxicity against B16-melanoma at a concentration of less than 100 μg/ml.

Experimental

Infrared (IR) spectra were recorded on a JASCO IR Report-100 spectrophotometer and UV spectra on a Shimadzu UV-visible recording spectrophotometer, UV-260. ^{1}H-NMR spectra were measured with a JEOL GX-400 spectrometer. MS were measured on a JMS-AX505H mass spectrometer.

Electrolysis The electrolysis was performed by maintaining constant potential in a glassy carbon beaker (5 × 4.5 cm) as an anode and platinum wire tip as a cathode. The potentio-galvanostat was a Toho Technical Research Model 2001 and the supporting current electrode (SCE) was a Toa Electronics Ltd. Model HS-305DS.

Electrochemical Oxidation of Etoposide A solution of etoposide (58 mg) and sodium perchlorate (56 mg) in methanol (20 ml) was electrolyzed under constant potential (500 mV) until the current ceased (105 min) with stirring at room temperature. The mixture was concentrated under reduced pressure and the residue was dissolved in dichloromethane. The mixture was washed with water, dried and concentrated under reduced pressure. Chromatography of the residue on a column of C_{18}-silica gel, eluting with methanol–water, afforded three components, **4**, **5** and **6**, whose structures were confirmed by the ^{1}H-NMR and mass spectral data.

1,2-Didehydroetoposide (4) Yellow powder, mp 145—147 °C. IR (KBr): 3430, 2910, 1740 (lactone), 1600 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 213 (24400), 246 (20200), 342 (10400). ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, δ (ppm): 6.78 (1H, s, 5-H), 6.62 (1H, s, 8-H), 6.53 (2H, br, 2′,6′-H), 6.05 (2H, dd, J=1.3, 14.1 Hz, 14-H), 5.7 (1H, s, 4′-OH), 4.74 (1H, q, J=5.1 Hz, 7″-H), 4.69 (1H, d, J=4.3 Hz, 4-H), 4.62 (1H, t, J=9 Hz, 11-H), 4.56 (1H, t, J=9 Hz, 11′-H), 4.21 (1H, dd, J=4.7, 10.3 Hz, 6″$_{eq}$-H), 4.20 (1H, d, J=7.7 Hz, 1″-H), 3.70 (6H, s, 3′,5′-OCH_3), 3.64 (1H, m, 3-H), 3.63 (1H, t, J=9.0 Hz, 3″-H), 3.61 (1H, t, J=10.3 Hz, 6″$_{aq}$-H), 3.38 (1H, dd, J=8, 9 Hz, 2″-H), 3.33 (1H, t, J=9 Hz, 4″-H), 3.25 (1H, dt, J=4.7, 9.6 Hz, 5″-H), 3.15 (2H, br, 2″, 3″-OH), 1.38 (3H, s, 8″-CH_3). FAB-MS m/z: 587 $(M+H)^+$, 381 $(M-(glycoside-OH)+H)^+$.

4′-*O*-Demethyl-1,2,3,4-tetradehydro-4-dehydroxypodophyllotoxin (5) Colorless powder, mp 249—251 °C. IR (KBr): 3400, 2900, 1760 (lactone), 1600 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 214 (28500), 261 (44700), 312 (11200), 351 (5900). ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.69 (1H, s, 4-H), 7.20 (1H, s, 5-H), 7.14 (1H, s, 8-H), 6.57 (2H, s, 2′,6′-H), 6.08 (2H, s, 14-H), 5.64 (1H, s, 4′-OH), 5.38 (2H, s like, 11, 11′-H), 3.88 (6H, s, 3′,5′-OCH_3). FAB-MS m/z: 381 $(M+H)^+$.

1,2,3,4-Tetradehydroetoposide (6) Colorless solid, mp >290 °C. IR (KBr): 3400, 2950, 1760 (lactone), 1610 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 214 (17900), 257 (26500), 309 (5700), 351 (3700). ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ (ppm): 8.00 (1H, s, 8-H), 7.11 (1H, s, 5-H), 6.55 (2H, dd, J=1.8, 8.4 Hz, 2′, 6′-H), 6.09 (2H, dd, J=1.1, 4.1 Hz, 14-H), 5.98 (1H, s, 4′-OH), 5.41 (2H, dd, 11, 11′-H), 5.03 (1H, d, J=3.3 Hz), 4.87 (1H, d, J=7.3 Hz), 4.78 (1H, q, J=4.8 Hz, 7″-H), 4.36 (1H, d, J=2.9 Hz, 3″-OH), 4.09 (1H, dd, J=4.9, 10.3 Hz, 6″$_{eq}$-H), 3.87 (6H, s, 3′, 5′-OCH_3), 3.82 (1H, dt, J=3.3, 8.1 Hz, 3″-H), 3.61 (1H, t, J=10.3 Hz, 6″$_{aq}$-H), 3.76 (1H, dt, J=2.6, 9.1 Hz, 2″-H), 3.46 (1H, t, J=9.2 Hz, 4″-H), 3.23 (1H, dt, J=4.9, 9.6 Hz, 5″-H), 1.39 (3H, s, 8″-CH_3). FAB-MS m/z: 585 $(M+H)^+$, 397 $(M-glycocyl+H)^+$.

Investigation of Effects of Supporting Electrolytes and Potential on the Ratio of Products A solution of etoposide (58 mg) and an appropriate amount of electrolyte in methanol (20 ml) was electrolyzed under constant potential (500 or 250 mV) until the current ceased, with stirring at room temperature. The ratios of the products were determined by HPLC (column, SenshuPak SSC-ODS-262; mobile phase, 1/100 M pH 7.0 phosphate buffer : MeOH = 40 : 60; flow rate, 1 ml/min; retention times, **1**, 3.2 min, **4**, 5.8 min, **5**, 7.0 min, **6**, 5.2 min). The results are summarized in Table I.

References

1) J. J. M. Holthuis, W. J. Van Oort, F. M. G. M. Römkens, J. Renema, *J. Electroanal. Chem.*, **184**, 317 (1985); J. J. M. Holthuis, D. E. M. M. Vendrig. W. J. Van Oort, P. Zuman, *ibid.*, **220**, 101 (1987); P. Zuman, J. J. M. Holthuis, *Recl. Trav. Chim. Pays-Bas*, **107**, 403 (1988).
2) M. Broggini, C. Rossi, E. Benfenati, M. D'Incaluci, R. Fannelli, P. Gariboldi, *Chem.-Biol. Interact.*, **77**, 215 (1985).
3) J. A. Richards, D. H. Evans, *J. Electroanal. Chem.*, **81**, 171 (1977).
4) B. Kalyanaraman, J. Nemec, B. K. Sinha, *Biochemistry*, **28**, 4839 (1989).

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