Studies in Sugar Chemistry VII[1]. Glucuronides of Podophyllum Derivatives

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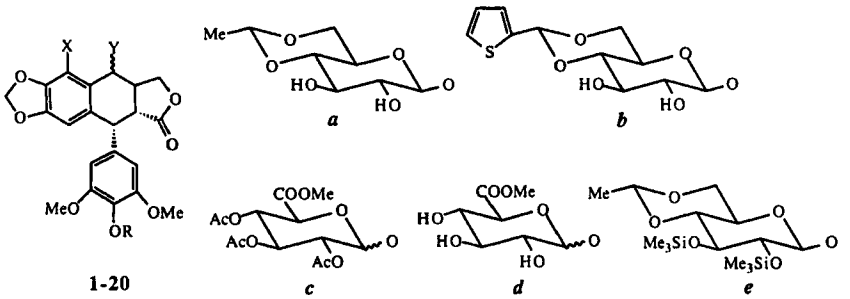
Summary

The antitumor activities of several glucuronide methyl esters of podophyllum derivatives were tested *in vitro* against two human tumor cell lines and their drug resistant sublines. The most active compound studied was methyl (4′-carbobenzoxy-4′-demethyl-epipodophyllotoxin-D-glucopyranoside)uronate **19**. Compound **19** was as potent in a colon carcinoma model and was twice as potent in a lung carcinoma model as etoposide **6**. *In vivo*, however, in a mouse leukemia P388 model, it had only marginal activity, with a maximum T/C% value of 125 at 37 mg/kg (iv).

Introduction

The antimitotic lignanes, podophyllotoxin **1**, 4′-demethyl-epipodophyllotoxin **2**, and α- and β-peltatines **3** and **4**, have been isolated from the North American plant *Podophyllum peltatum Linnaeus* and the Indian variety *Podophyllum emodi Wallich*. A mixture of these compounds, called podophyllum resin[2], has limited clinical use as a caustic for warts. Two clinically useful anticancer drugs, etoposide **6** and teniposide **7**, are obtained[3], however, when **2** is coupled with 4,6-*O*-ethylidene- and 4,6-*O*-2-thenylidene-β-D-glucose respectively.

The environmental pH of some tumor tissues is lower than physiological pH[4], and in addition, some of these tissues are rich in the enzyme β-glucuronidase which displays an optimal activity at pH ca. 5[5]. Based on these facts, various investigations[6] have attempted to use glucuronides of anticancer drugs as selective tumor targeting entities. These glucuronides, which possess very low systemic toxicity, are expected[7] to undergo preferred localized cytotoxic activation at the tumor site upon hydrolysis by β-glucuronidase. More recent publications describe activation of glucuronide prodrugs by antibody-directed enzyme prodrug therapy[8]. In this report, we describe the preparation of glucuronides **15–19** (Fig. 1), which were synthesized in order to examine their antitumor activity and to evaluate the effect of glucuronidation on the toxicity of podophyllum derivatives.



Compound	R	X	Y
1 Podophyllotoxin	Me	H	OH (α)
2 4′-Demethylepipodophyllotoxin	H	H	OH (α)
3 α-Peltatin	H	OH	H
4 β-Peltatin	Me	OH	H
5 4′-CBZ-epipodophyllotoxin	CBZ	H	OH (β)
6 Etoposide	H	H	*a* (β)
7 Teniposide	H	H	*b* (β)
10	Me	H	*c*
11	H	H	*c*
12	H	*c*	H
13	Me	*c*	H
14	CBZ	H	*c*
15	Me	H	*d*
16	H	H	*d*
17	H	*d*	H
18	Me	*d*	H
19	CBZ	H	*d*
20	Me_3Si	H	*e*

Fig. 1. Structural formulae of podophyllotoxin derivatives.

Chemistry

Glucuronidation of compounds **1–5** was carried out by the methods of Kuhn[9] and Schmidt[10] using the α,β-1-OH[11] or β-1-O-C=(NH)-CCl_3[11] derivatives (**8** and **9**) as substrates (Figs. 2 and 3). In most cases the isolated glucuronides **10–14** were mixtures of α- and β-anomers, as shown by the ^{1}H-NMR spectra of the mixtures, in which the α-anomers displayed J = 4 Hz and the β-anomers J = 8 Hz for the H-1 proton. Fractional crystallization afforded the individual anomers.

Compound **16** was prepared by either deacetylation of **11** or hydrogenolysis of the carbobenzoxy group of **19** (Fig. 3). In the course of the glucuronidation of **5** with **8** or **9** to give **14**, a small amount of **21**, the *neo*-isomer[12] of **5**, was isolated. While peltatins **3** and **4** underwent satisfactory glucuronidation with sugar **9**, with **8**, only poor yields were obtained. The failure of glucuronidation of the 4′-phenolic group of α-peltatin may be

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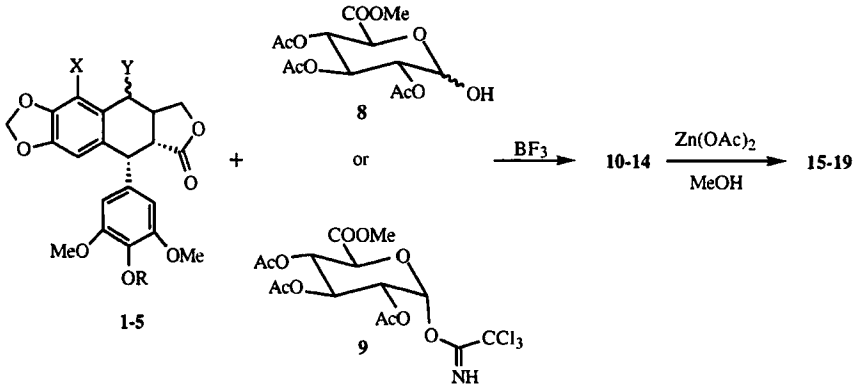

Fig. 2. General synthesis of podophyllotoxin glucuronide derivatives.

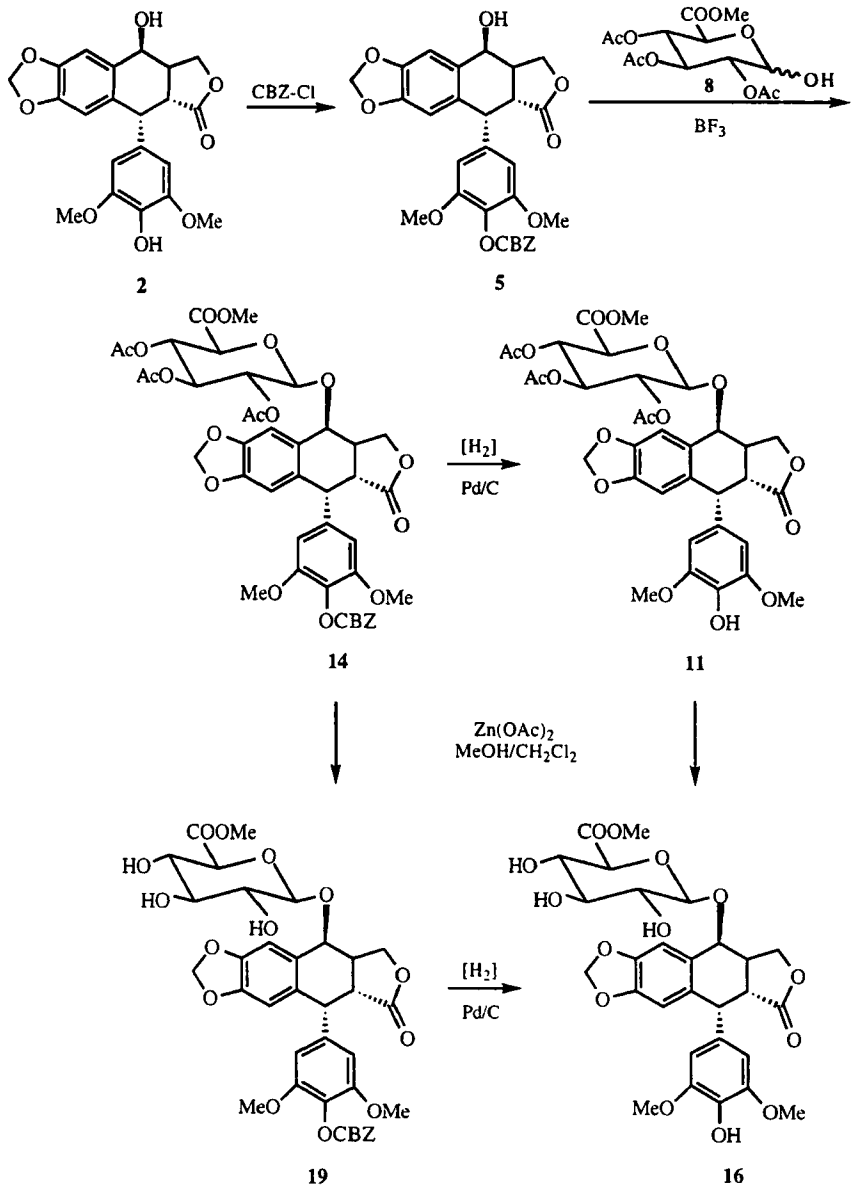

Fig 3. Synthesis of most active glucuronide derivatives **19** and **16.**

attributed to steric hindrance. This is in agreement with results obtained on model compounds. For instance, guaiacol was readily glucuronidated with **9**, but all attempts to carry out the analogous reaction with 2,6-dimethoxyphenol were unsuccessful. An unexpected trans-glycosidation took place upon attempted glucuronidation of etoposide **6** or its tris-trimethylsilylated derivative **20**. The only glucuronidation product from **6** or **20** isolated using either **8** or **9** was compound **11**. Deacetylation of glucuronides **10–14** was carried out in the presence of $Zn(OAc)_2$ in a MeOH-CH_2Cl_2 mixture due to poor solubility of the substrates in pure MeOH.

Biological Evaluation

In Vitro Cytotoxicity in the National Cancer Institute Leukemia Screen (3PS31) – Glucuronides **5–19,** were evaluated at the National Cancer Institute by using the leukemia screen 3PS31. While **15, 16,** and **18** were inactive at concentration ranges below 25–6.5 mg/kg, glucuronide **19** showed T/C% values of 127 at 12.5 mg/kg, 118 at 3.12 mg/kg, 107 at 1.56 mg/kg, and 102 at 0.78 mg/kg. The poor cytotoxic activity of **15, 16,** and **18** may be understood in terms of their high hydrophilicity. Despite these compounds being methyl ester derivatives of glucuronides, apparently, they are readily excreted, as are most other glucuronide metabolites. In the case of **19**, the presence of the 4′-carbobenzoxy protective group appears to make the molecule more lipophilic and hence better able to penetrate into the tumor cells, thus providing higher cytotoxic activity.

In Vitro Cytotoxicity – The podophyllotoxin analog **19** was tested (see experimental part) against two human tumor cell lines and their drug resistant sublines (Table 1). This compound was as potent as etoposide in the colon carcinoma HCT116 and twice as potent than etoposide in the lung carcinoma cell line A549. In addition, **19** was not cross resistant with etoposide in the etoposide resistant sublines

Table 1. *In Vitro* Cytotoxicity of **19** (BMY41340).

	IC_{50} (μ M) [a]				
	Cell lines [b]				
	HCT116	HCT116(VM)46	HCT116 (VP)35	A549	A549(VP)28
19	1.08	1.09	0.86	2.13	2.08
Etoposide	1.01	3.72	13.3	4.08	15.6

[a] Cytotoxicity values determined after 72 h drug exposure by XTT assay.
[b] Cell lines: HCT-116 human colon; HCT-116(VM)46 and HCT-116(VP)35 human colon etoposide resistant; A549 human lung; A549(VP)28 human lung etoposide resistant.

Table 2. Summary of *in vivo* antitumor activity of **19** (BMY41340).

Tumor Model (site)	Schedule	Optimal Dose [a] (mg/kg/inj)	%T/C
P388 (ip)	ip q4dX2; 1	150	125
P388 (iv)	ip q4dX2; 5	37	125

[a] Optimal dose was also the maximal tolerated dose

HCT116(VP)35 and A549(VP)28, nor in the multidrug resistance cell line HCT116(VM)46. These data suggest that this compound is not a substrate for the P-glucoprotein efflux pump nor does it interact with topoisomerase II.

Thus, we find that in tumor models in which etoposide **6** is known to be very active the podophyllotoxin analog **19**, for both ip and iv injection, at its maximally tolerated dose (150 mg/kg/inj for ip and 37 mg/kg/ink for iv) gave an optimal result %T/C of 125%. We also note that although compound **15**, a methyl ester of a typical glucuronide, displayed poor antitumor activity compared to etoposide, it did show a very low degree of systemic toxicity [13].

Acknowledgment

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Experimental Part – Chemistry

General Remarks

^{1}H NMR spectra were obtained in $CDCl_3$ on Bruker AM-300 and AM-270 spectrometers. Mass spectra were obtained on a Finnigan 4021 spectrometer (CI = chemical ionization, using CH_4 as carrier gas; EI = electron ionization). All reactions were carried out in anhydrous conditions under N_2, using freshly distilled solvents. Progress of the reactions was monitored by TLC on silica gel (Merck, Art. 5554) eluted with EtOAc/hexane mixtures, spraying with 1% vanillin/1% H_2SO_4 in MeOH and developing on a hot plate. Flash chromatography was carried out on silica gel (Riedel-de Haen, 32–63 μm).

Methyl (1-O-trichloroacetimidoyl-2,3,4-tri-O-acetyl-α-D-glucopyranoside)-uronate (**9**)

To NaH (55–60% dispersion in oil, 0.114 g, 2 mmol), washed with petroleum ether, covered with CH_2Cl_2 (5 mL) and Cl_3CCN (3 mL, 30 mmol), at 0 °C, 2,3,4-tri-*O*-acetyl-D-glucopyranuronate **8**[1,4] (1 g, 3 mmol) in CH_2Cl_2 (5 mL), was added dropwise. When **8** could not be detected by TLC, the precipitate was filtered and washed with CH_2Cl_2. The combined organic phase was concentrated and the residue was chromatographed on silica gel and eluted with 1:5 ether-CH_2Cl_2, to afford **9** (0.7 g, 50% yield) that was recrystallized from *i*-PrOH, mp 106–108 °C.– ^{1}H NMR: δ 8.76 (1H, NH), 6.63 (d, 1H, H-1, $J_{1,2}$ = 3.5 Hz), 5.64 (t, 1H, H-3, J = 9.8 Hz), 5.26 (t, 1H, H-4, J = 9.9 Hz), 5.14 (dd, 1H, H-2, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.45 Hz), 4.49, (d, 1H, H-5, $J_{4,5}$ = 9.9 Hz), 3.75 (s, 3H, Me), 2.05 (s, 6H, Ac), 2.02 (s, 3H, Ac).– MS (CI) *m/z* = 478 [MH^+], 442 [MH^+ – HCl], 400 [442 – CH_2CO], 418 [M^+ – AcOH], 376 [418 – CH_2CO], 358 [376 – H_2O], 333 [MH^+ – $CNHCCl_3$], 317 [MH^+ – $OCNHCCl_3$], 257 [317 – AcOH], 215 [257 – CH_2CO], 197 [257 – AcOH], 169 [317 – 2AcOH], 155 [215 – AcOH], 127 [155 – CO].

2,3,4′-Tris-trimethylsilyletoposide (**20**)

To a stirred solution of **6** (100 mg, 0.17 mmol) in pyridine (10 mL) were added $(Me_3Si)_2NH$ (2 mL, 9.7 mmol) and Me_3SiCl (1 mL, 7.9 mmol), and the mixture was heated to 75–80 °C for 10 min. The precipitate was filtered under N_2 and the filtrate concentrated. The residue was washed with petroleum ether to give **20** (123 mg, 90%), mp 228–230 °C.– MS (EI) *m/e* = 804 [M^+], 789 [M^+ – Me], 732 [M^+ – CH_2CO_2], 471 [M^+ – 333 $C_{14}H_{29}O_5Si_2$], 455 [M^+ – 349 $C_{14}H_{29}O_6Si_2$], 411 [455 – CO_2], 259 [349 – Me_3SiOH], 185 [259 – $MeCHO_2$].

General procedures for glucuronidation with **8** *or* **9**

To a solution of a derivative **1–5** (2.5 mmol), in $ClCH_2CH_2Cl$/THF (3–5 mL) was added **8** (1.23 g, 3.7 mmol) or **9** (1.76 g, 3.7 mmol). The stirred mixture was cooled in an ice bath, and BF_3·ether (0.9 mL, 7 mmol) was added dropwise. The mixture was stirred at room temperature until complete disappearance of **1–5**, and then pyridine (1 mL) was added. The mixture was quenched with water and the organic phase was dried and evaporated. The residues were crystallized or chromatographed to give **10–14** respectively.

Methyl (2,3,4-tri-O-acetyl-epipodophyllotoxin-D-glucopyranoside)uronate (**10**)

Compound **10** was obtained from **1** in 67% yield. Fractional recrystallization from *i*-PrOH provided the individual anomers. β-*Anomer*: mp 230–231 °C. ^{1}H NMR: δ: 6.79 (s, 1H, H^8), 6.57 (s, 1H, H^5), 6.23 (s, 2H, $H^{2'}$ + $H^{6'}$), 6.00 (dd, 2H, CH_2), 5.3–5.2 (m, 2H, H^{11} + $H^{11'}$), 5.01 (m, 1H, H-3), 4.95 (d, 1H, H^4), 4.79 (d, 1H, H-1), 4.59 (d, 1H, H^1), 4.47 (dd, 1H, H-4), 4.29 (dd, 1H, H^2), 4.05 (m, 1H, H-5), 3.80 (s, 3H, 4′-OMe), 3.80 (s, 3H, COOMe), 3.73 (s, 6H, 3′-OMe + 5′-OMe), 3.17 (dd, 1H, H^2), 2.97–2.82 (m, 1H, H^3), 2.04 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.89 (s, 3H, Ac). α-*Anomer*: mp 185–188 °C.– ^{1}H NMR: δ 6.92 (s, 1H, H^8), 6.55 (s, 1H, H^5), 6.24 (s, 2H, $H^{2'}$ +

$H^{6'}$), 5.97 (dd, 2H, CH_2), 5.39 (dd, 1H, H-3), 5.34 (d, 1H, H-1), 5.18 (dd, 1H, H^4), 5.04 (dd, 1H, H-2), 4.76 (H-4, d), 4.68 (d, 1H, H^1), 4.29–4.25 (m, 2H, $H^{11} + H^{11'}$), 4.01 (d, 1H, H-5), 3.80 (s, 3H, 4'-OMe), 3.77 (s, 3H, COOMe), 3.74 (s, 6H, 3'-OMe + 5'-OMe), 3.46 (dd, 2H, H^2), 2.99–2.85 (m, 1H, H^3), 2.095 (s, 3H, Ac), 2.017 (s, 3H, Ac), 1.986 (s, 3H, Ac). MS (CI) *m/z* = 759 [$M^+ + C_2H_5$], 731 [MH^+], 185 [229 – CO_2], 317 [334 – OH], 257 [317 – AcOH], 197 [257 – AcOH], 169 [317 – 2AcOH], 155 [197 – CH_2CO].– Anal. $C_{35}H_{38}O_{17}$ (730.7).

Methyl (2,3,4-tri-O-acetyl-α-peltatin-D-glucopyranoside)-uronate (**12**)

Compound **12** was obtained from **3** as a mixture of α- and β-anomers. β-*Anomer* ^{1}H NMR: δ 6.33 (s, 2H, $H^{2'} + H^{6'}$), 6.32 (s, 1H, H^8), 5.94 (br s, 2H, CH_2), 4.60–4.47 (m, 5H, H-1, H-2, H-3, H-4, and H^1), 3.95–3.6 (m, 3H, H-5, $H^{11} + H^{11'}$), 3.80 (s, 3H, 4'-OMe), 3.76 (s, 3H, COOMe), 3.72 (s, 6H, 3'-OMe + 5'-OMe), 3.19 (dd, 1H, H-2), 2.52–2.3 (m, 2H, H-3, H-4), 2.07 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac).– α-*Anomer* ^{1}H NMR: δ 6.36 (s, 2H, $H^{2'} + H^{6'}$), 6.33 (s, 1H, H^8), 5.94 (br s, 2H, CH_2), 5.54 (d, 1H, H-1), 5.39–5.25 (m, 3H, H-2 + H-3 + H-4) 4.6–4.47 (m, H-1, buried under the m of H-1, H-2, H-3, and H-4 of the β-anomer), 4.12 (d, 1H, H-5), 3.9–3.6 (m, 2H, $H^{11} + H^{11'}$), 3.80 (s, 3H, 4'-OMe), 3.76 (s, 3H, COOMe), 3.74 (s, 6H, 3'-OMe + 5'-OMe), 3.29 (dd, 1H, H^2), 2.70–2.64 (m, H^3, H^4), 2.07 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac).– MS (EI) *m/z* = 759 [M – $C_2H_5^+$], 731 [MH^+], 671 [MH^+ – AcOH].

Methyl (2,3,4-tri-O-acetyl-β-peltatin-D-glucopyranoside)-uronate (**13**)

Compound **13** was obtained from **4** in 45% yield as a mixture of α- and β-anomers (ratio ca. 3:2), and was recrystallized from CH_2Cl_2; mp 248–250 °C. β-*Anomer* ^{1}H NMR: δ 6.34 (s, 2H, $H^{2'} + H^{6'}$), 6.20 (s, 1H, H^8), 5.95 (br s, 2H, CH_2), 5.55 (d, 1H, H-1), 5.45–5.24 (m, 3H, H-2, H-3, H-4) 4.61-4.43 (H-1, buried under the m of H-1, H-2, H-3, and H-4 of the β-anomer), 4.0–3.7 (m, 3H, H-5 + $H^{11} + H^{11'}$), 3.77 (s, 6H, 3'-OMe + 5'-OMe), 3.72 (s, 3H, COOMe) 3.21 (dd, 1H, H^2) 2.5–2.3 (m, 2H, H^3, H^4), 2.07 (s, 3H, Ac) 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac).– α-*Anomer* ^{1}H NMR: δ 6.36 (s, 2H, $H^{2'} + H^{6'}$), 6.33 (s, 1H, H^8), 5.95 (br s, 2H, CH_2), 5.55 (d, 1H, H-1), 5.45–5.24 (m, 3H, H-2, H-3, H-4) 4.61–4.43 (H-1, buried under the m of H-1, H-2, H-3, and H-4 of the α-anomer), 4.16 (d, 1H, H-5), 3.9–3.6 (m, 2H, $H^{11} + H^{11'}$), 3.77 (s, 6H, 3'-OMe + 5'-OMe), 3.72 (s, 3H, COOMe) 3.31 (dd, 1H, H^2) 2.7–2.57 (m, 2H, H^3, H^4), 2.07 (s, 3H, Ac) 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac).– MS (EI) *m/z* = 745 [M – C_2H_5+], 717 [MH^+], 657 [MH^+ – AcOH], 597 [657 – AcOH], 537 [597 – AcOH], 503 [563 – AcOH], 471 [503 – MeOH], 443 [503 – AcOH], 429 [471 – CH_2CO], 383 [400 – OH].

Methyl (2,3,4-tri-O-acetyl-4'-carbobenzoxy-4'-demethyl-epi-podophyllotoxin-D-glucopyranoside)uronate (**14**) *and 4'-carbobenzoxy-4'-demethyl-neopodophyllotoxin* (**21**)

Compound **14** was obtained from **5** in 68% yield as a mixture of α- and β-anomers (ratio ca. 2:1) and was recrystallized from *i*-PrOH, mp 208–210 °C.– β-*Anomer* ^{1}H NMR: δ 7.51–7.36 (m, 5H, Ph), 6.77 (s, 1H, H^8), 6.56 (s, 1H, H^5), 6.26 (s, 2H, $H^{2'} + H^{6'}$), 5.99 (dd, 2H, CH_2), 5.3–5.2 (m, 2H, $H^{11} + H^{11'}$), 5.25 (s, 2H, PhCH_2), 5.01 (m, 1H, H-3), 4.94 (d, 1H, H^4), 4.79 (d, 1H, H-1), 4.62 (d, 1H, H^1), 4.47 (dd, 1H, H-4), 4.28 (m, 1H, H-2), 4.04 (m, 1H, H-5), 3.79 (s, 3H, COOMe), 3.67 (s, 6H, 3'-OMe + 5'-OMe), 3.18 (dd, 1H, H^2), 2.95–2.8 (m, 1H, H^3), 2.04 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.88 (s, 3H, Ac).– α-*Anomer* ^{1}H NMR: δ: 7.51–7.36 (m, 5H, Ph), 6.92 (s, 1H, H^8), 6.55 (s, 1H, H^5), 6.26 (s, 6H, $H^{2'} + H^{6'}$), 5.97 (dd, 2H, CH_2), 5.38 (dd, 1H, H-3), 5.33 (d, 1H, H-1), 5.25 (s, 2H, Ph-CH_2), 5.04 (dd, 1H, H-2), 5.18 (dd, 1H, H^4), 4.74 (d, 1H, H-4), 4.72 (d, 1H, H^1), 4.32–4.24 (m, 2H, $H^{11} + H^{11'}$), 3.99 (d, 1H, H-5), 3.77 (s, 3H, COOMe), 3.68 (s, 6H, 3'-OMe + 5'-OMe), 3.47 (dd, 1H, H^2), 2.95–2.8 (m, 1H, H^3), 2.095 (s, 3H, Ac), 2.017 (s, 3H, Ac), 1.986 (s, 3H, Ac).– MS (EI) *m/e* = 715 [M^+ – $PhCH_2OCO$], 655 [715 – AcOH].– Anal. $C_{42}H_{42}O_{18}$ (834.8). The *i*-PrOH-insoluble material was identified as *4'-carbobenzoxy-4'-demethyl-neopodophyllotoxin* (**21**) mp 270–272 °C.– ^{1}H NMR: δ 7.42–7.3 (m, 5H, Ph), 6.85 (s, 1H, H-8), 6.56 (s, 1H, H-5), 6.23 (s, 2H, $H^{2'} + H^{6'}$), 6.00 (dd, 2H, CH_2), 5.25 (s, 2H, PhCH_2), 4.95 (d, 1H, H-4), 4.64 (d, 1H, H-1), 3.94 and 3.61 (two dd, 2H, CH_2OH), 3.65 (s, 6H, 3'-OMe + 5'-OMe), 3.27 (dd, H1, H-2, dd), 2.9–2.8 (m, 1H, H-3).– MS (EI) *m/e* = 490 [M^+ – CO_2], 399 [M^+ – $PhCH_2OCO$], 355 [399 – CO_2], 337 [381 – CO_2], 324 [355, CH_2OH], 306 [337 – CH_2OH], 202 [246 – CO_2], 185 [202 – OH], 154 [MH^+ – 246 – $PhCH_2OCO$], 108 [C_7H_7OH], 91 [C_7H_7].

Methyl (2,3,4-tri-O-acetyl-4'-demethyl-epipodophyllotoxin-D-glucopyranoside)uronate (**11**) *from* **14**

A solution of **14** (0.51 g, 0.6 mmol) in acetone-EtOH (1:1, 5 mL) and AcOH (0.02 mL), was hydrogenated over 10% Pd/C (0.2 g) at atmospheric pressure until starting material could not be detected by tlc. The catalyst was filtered and the filtrate was evaporated to dryness, to give **11** (0.27 g, 70% yield), as a mixture of α- and β-anomers of mp 257–260 °C. β-*Anomer* ^{1}H NMR: δ 6.81 (s, 1H, H^8), 6.57 (s, 1H, H^5), 6.28 (s, 2H, $H^{2'} + H^{6'}$), 5.99 (dd, 2H, CH_2), 5.40 (m, 1H, H-3), 5.3–5.2 (m, 2H, $H^{11} + H^{11'}$, m), 4.96 (dm 1H, H^4, 4.81 (d, 1H, H^1), 4.60 (dd, 1H, H-1), 4.47 (dd, 1H, H-2), 4.02 (d, 1H, H-5), 3.80 (s, 3H, COOMe), 3.77 (s, 6H, 3'-OMe + 5'-OMe), 3.18 (dd, 1H, H^2), 2.98–2.82 (m, 1H, H^3), 2.11 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.00 (s, 3H, Ac).– α-*Anomer* ^{1}H NMR: δ 6.94 (s, 1H, H^8), 6.57 (s, 1H, H^5), 6.28 (s, 2H, $H^{2'} + H^{6'}$), 5.98 (CH_2, dd), 5.40 (dd, 1H, H-3), 5.36 (d, 1H, H-1), 5.06 (dd, 1H, H-2), 5.19 (dd, 1H, H-4), 4.76 (d, 1H, H^4), 4.69, (d, 1H, H^1), 4.29–4.25 (m, 2H, $H^{11} + H^{11'}$, m), 4.02 (d, 1H, H-5), 3.77 (s, 9H, COOMe + 3'-OMe + 5'-OMe), 3.46 (dd, 1H, H^2), 2.98–2.82 (m, 1H, H^3), 2.04 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.88 (s, 3H, Ac).– MS (EI) *m/e* = 716 [M^+], 588 [M^+ – 2MeCO – CH_2CO].

Methyl (2,3,4-tri-O-acetyl-1-(2-methoxyphenyl)-D-glucopyranoside)-uronate

This compound was prepared from **9** and guaiacol, mp 174–175 °C. ^{1}H NMR: δ 7–6.8 (m, 4H, Ar), 5.34–5.29 (m, 3H, H-2, H-3, H-4), 5.15 (d, 1H, H-1), 4.18 (m, 1H, H-5), 3.81 (s, 3H, OMe), 3.74 (s, 3H, COOMe), 2.08 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.03 (s, 3H, Ac).– MS (CI) *m/z* = 381 [M – CO_2Me], 317 [M – $C_7H_7O_2$].

General procedure for removal of acetyl protecting groups

To a suspension of **10–14** (1 mmol) in anhydrous MeOH (5 mL) was added CH_2Cl_2 until a clear solution was obtained upon reflux. Anhydrous $Zn(OAc)_2$ (1 mmol if no free OH was present or 2 mmol if a free OH was present in **10–14**) was added and the mixture was refluxed for 24 h. After the addition of AcOH (0.05 mL) the volatiles were removed and the residue was dissolved in $CHCl_3$/*i*-PrOH (4:1, 30 mL), washed with water (2x3 mL), dried and evaporated. The residue was washed with a suitable solvent, recrystallized or chromatographed to give **15–19** respectively.

Methyl (epipodophyllotoxin-D-glucopyranoside)uronate (**15**)

Compound **15** was obtained from **10** (75% yield) upon washing the residue with $CHCl_3$, mp 259–260 °C. ^{1}H NMR: δ 7.00 (s, 1H, H^8), 6.49 (s, 1H, H^5), 6.25 (s, 2H, $H^{2'} + H^{6'}$), 5.95 (dd, 2H, CH_2), 5.08 (d, 1H, H-1), 4.81 (dd, 1H, H^{11} or $H^{11'}$), 4.75 (d, 1H, H^4), 4.60 (d, 1H, H^1), 4.27 (dd, 1H, H^{11} or $H^{11'}$), 3.99 (m, 1H, H-5), 3.86 (s, 3H, 4'-OMe), 3.79 (s, 3H, COOMe), 3.73 (s, 6H, 3'-OMe + 5'-OMe), 3.7–3.6 (m, 3H, H-2 + H-3 + H-4), 3.49 (dd, 1H, H^2), 2.92–2.86 (m, 1H, H^3).– MS (EI) *m/e* = 604 [M^+].

Methyl (4'-demethyl-epipodophyllotoxin-D-glucopyranoside)uronate (**16**)

Compound **16** was obtained by $Zn(OAc)_2$ catalyzed methanolysis of **11** (67% yield) or hydrogenolysis of **19** (98% yield) and was recrystallized from EtOH, mp 270–272 °C. ^{1}H NMR: δ 6.88 (s, 1H, H^8), 6.45 (s, 2H, $H^{2'} + H^{6'}$), 6.39 (s, 1H, H^5), 5.88 (dd, 2H, CH_2), 5.13 (d, 1H, H-1), 4.70 (d, 1H, H^4), 4.5–4.4 (m, 2H, $H^{11} + H^{11'}$), 4.26 (d, 1H, H^1), 3.82 (s, 6H, 3'-OMe + 5'-OMe), 3.75–3.5 (m, 4H, H-2, H-3, H-4, H-5), 3.65 (s, 3H, COOMe), 3.28 (dd, 1H, H^2), 3.07–3.02 (m, 1H, H^3).

*Methyl (α-peltatine-D-glucopyranoside)uronate (**17**)*

Compound **17** was obtained form **12** in 50% yield and was purified by chromatography, mp 273–274 °C. ^{1}H NMR: δ 6.38 (s, 1H, H^8), 6.35 (s, 2H, $H^{2'}$ + $H^{6'}$), 5.96 (br s, 2H, CH_2), 5.25 (d, 1H, H-1), 4.59 (br s, 1H, H^1), 4.53–4.452 (m, 2H, H^{11} + $H^{11'}$), 4.1–3.5 (m, 4H, H-2, H-3, H-4, H-5), 3.80 (s, 3H, COOMe), 3.75 (s, 6H, 3′-O Me + 5′-OMe), 3.34 (br d, 1H, H^2), 2.7–2.4 (m, 2H, H^3, H^4).– MS (CI) *m/z* = 589 [M^+ – 1], 202 [246 – CO_2], 200 [246 – CH_2OCO].

*Methyl (β-peltatine-D-glucopyranoside)uronate (**18**)*

Compound **18** was obtained form **13**. ^{1}H NMR: δ 6.37 (s, 1H, H^8), 6.35 (s, 2H, $H^{2'}$ + $H^{6'}$), 5.96 (dd, 2H, CH_2), 5.24 (d, 1H, H-1), 4.6 (d, 1H, H^1), 4.5–4.42 (m, 2H, H^{11} + $H^{11'}$), 3.96–3.7 (m, 3H, H-2, H-3, H-4), 3.81 (s, 3H, 4′-OMe), 3.80 (s, 3H, COOMe), 3.78 (s, 6H, 3′-O Me + 5′-OMe), 3.36 (br d, 1H, H^2), 2.70–2.45 (m, 2H, H^3, H^4).– MS (CI) *m/z* = 604 [M^+], 586 [M^+ – H_2O], 572 [M^+ – MeOH], 369 [414 – HCO_2].

*Methyl (4′-carbobenzoxy-4′-demethyl-epipodophyllotoxin-D-glucopyranoside)uronate (**19**)*

Compound **19** was obtained from **14** and was recrystallized from EtOH (0.58 g, 46% yield), mp 229–230 °C. ^{1}H NMR: δ 7.45–7.32 (m, 5H, Ph), 7.04 (s, 1H, H^8), 6.49 (s, 1H, H^5), 6.25 (s, 2H, $H^{2'}$ + $H^{6'}$), 5.94 (dd, 2H, CH_2), 5.23 (s, 2H, PhCH_2, s), 5.09 (d, 1H, H-1), 4.30–4.13 (m, 2H, H^{11} + $H^{11'}$), 4.75 (d, 1H, H^4), 4.60 (d, 1H, H^1), 3.93 (m, 1H, H-5), 3.86 (s, 3H, COOMe), 3.65 (s, 6H, 3′-O Me + 5′-OMe), 3.7–3.6 (m, 3H, H-2, H-3, H-4), 3.42 (dd, 1H, H^2), 2.94–2.70 (m, 1H, H^3).– MS (EI) *m/e* = 517 [M^+ – X′], 471 [517 – OCH_2O], 443 [471 – CO], 415 [443 – CO], 383 [517 – $PhCH_2OCO$], 353 [381 – CO], 337 [381 – CO_2].– Anal. $C_{36}H_{36}O_{16}\cdot 2H_2O$.

Experimental Part – Biology

In Vitro Cytotoxicity – Cell Culture

The human colon carcinoma cell line HCT116 and the human lung carcinoma cell line A549, as well as the sublines discussed below, were maintained in McCoy's medium containing 10% fetal bovine serum and 0.05 M HEPES buffer[14]. Resistant sublines of these parental cell lines were maintained in the same medium. An etoposide resistant subline, HCT116(VP)35, whose resistance is likely due to low levels of topoisomerase II, was derived by stepwise selection in etoposide. A multidrug resistant subline HCT116(VM)46, was selected for growth in teniposide (VM-26) and expresses the multidrug resistance phenotype including overexpression of the drug efflux protein P-glycoprotein and resistance to a number of lipophilic anticancer compounds such as etoposide and vinblastine. A second etoposide resistant subline, A549(VP)28, which has low levels of topoisomerase II was selected for growth in etoposide.

Determination of In Vitro Cytotoxicity in Tissue Culture Cells

Cytotoxicity was assessed in human carcinoma cell lines by XTT 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetr azolium hydroxide assay[15]. Cells were plated at 4000 cells/well in 96 well microtiter plates; 24 h later drugs were added and serial diluted. The cells were incubated at 37 °C for 72 h at which time the tetrazolium dye, XTT, was added. A dehydrogenase enzyme in live cells reduces the XTT to a form that absorbs light at 450 nm, enabling quantitative spectrophotometric determination of the number of cells. The cytotoxicity results are expressed as an IC_{50} which is the drug concentration required to inhibit cell proliferation (as measured by the absorbance at 450 nm) to 50% of that of untreated control cells.

*In Vivo Antitumor Studies with the Podophyllotoxin Analog **19** (BMY41340)*

For *in vivo* evaluation of antitumor activity $1x10^6$ P388 mouse leukemia cells were implanted in the intraperitoneal (ip) cavity of (BALB/c X DBA/2)F_1 (CDF_1) or (C57/BL6 X DBA/2)F_1 (BDF_1) mice[16]. Glucuronide **19** was dissolved in DMSO, diluted with saline to the needed concentrations, and injected intraperitoneally. Injections were performed on the first day after tumor implantation and every fourth day thereafter for two treatments (q4dX2:1). Increases in life span were reflected by the median survival time of treated (T) vs. control (C) groups for which a %T/C was calculated. Antitumor activity was also evaluated in P388 cells ($1x10^6$) implanted intravenously (iv) in mice. The injection schedule and route were the same as for the ip P388 tumor models, but treatment initiation was delayed until day 5 post-leukemia implants. Results are expressed as %T/C. For both the ip and the iv the implanted P388 tumor models, a T/C of 3125% was considered an active result.

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