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NOVEL LIGNAN DERIVATIVES AS SELECTIVE INHIBITORS OF DNA TOPOISOMERASE II

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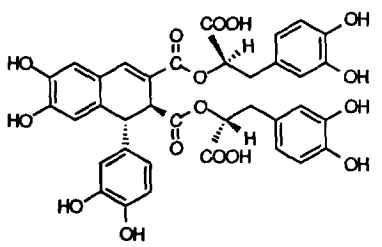
Abstract: Rabdosiin (**1**) and its isomer (**2**), isolated from *Arnebia euchroma,* were potent non-selective inhibitors of mammalian DNA topoisomerases *in vitro*. Evaluation of synthetic analogs led to the discovery of **3 – 5** as selective inhibitors of topoisomerase II. Unlike etoposide, which inhibits by preventing the DNA rejoining process, compounds **1 – 5** did not induce DNA breakage in either cellular or *in vitro* assay systems.

DNA topoisomerase II is involved in a number of vital cellular processes, including DNA replication, transcription, and chromosome segregation.[1] The catalytic mechanism is well understood as are the actions of several antitumor drugs that inhibit the enzyme, including the natural product derivative etoposide (VP-16).[2] A type I topoisomerase found in mammalian cells is also considered to be a viable target for cancer chemotherapeutic agents.[2] Our interest is to discover novel inhibitors as lead structures for drug development by isolating active constituents from medicinal plants. Using various screening strategies, certain tannins were recently identified as potent topoisomerase inhibitors with an unusual mechanism of action.[3-6]

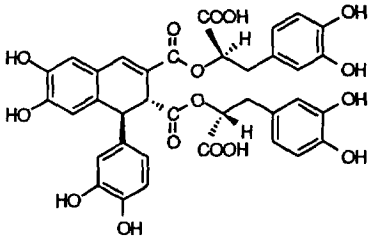
In the course of our continuing search for novel topoisomerases inhibitors from natural products, the aqueous acetone extract of *Arnebia euchroma* (Boraginaceae) was found to exhibit a potent inhibitory effect. Subsequent fractionation of the active fraction by MCI-gel CHP20P, Sephadex LH-20, ODS, and Toyopearl HW40F chromatography has resulted in the isolation of two phenolic compounds **1** and **2**. Compounds **1** and **2** were identified as rabdosiin and its isomer, respectively, by comparison of their physical and spectral data with those described in the literature.[7,8] Biological evaluation of these compounds showed that compounds **1** and **2** were non-selective inhibitors *in vitro,* like the active tannins evaluated to-date. Compounds **1** and **2** both possess a lignan skeleton, which is partially superimposable with the topoisomerase II inhibitor, VP-16. This observation prompted our preparation of simple analogs of these lignan compounds, and evaluation of their inhibitory effect against topoisomerases, as well as their ability to induce protein-DNA complex formation.

The lignan derivatives were prepared by oxidative coupling of caffeic acid derivatives by $FeCl_3$.[9] Thus, on oxidation with $FeCl_3$, methyl caffeoate gave a racemic compound **3**,[7] while similar oxidation of chlorogenic acid furnished diastereoisomeric lignans **4** and **5**.[10] On the other hand, similar oxidation of caffeic acid afforded a dilactone (**6**), which was subsequently treated with aqueous HCl to yield the dicarboxylic acid (**7**).

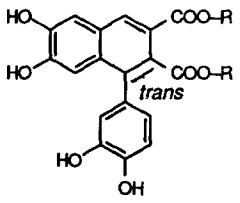
The structures of these compounds were established by spectral examinations and/or comparison of the spectral data with those described in the literature.[7]



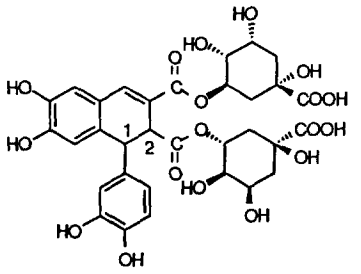
1



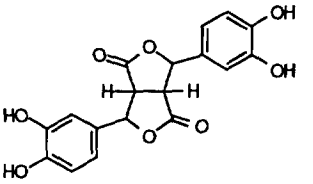
2



3 R=CH_3
7 R=H



4 1(*R*), 2(*S*)
5 1(*S*), 2(*R*)



6

Compounds **1** – **7** were evaluated side-by-side as inhibitors of DNA unknotting (type II-mediated) and ATP-independent DNA relaxation (type I-mediated) activities *in vitro*[11] (Table 1). The plant isolates (**1** and **2**) and two synthetic derivatives, **6** and **7**, were effective against both enzymes, whereas the synthetic lignan derivatives, **3** – **5**, were catalytic inhibitors with selectivity for topoisomerase II. Compounds **1** – **5** were also compared with etoposide in mechanistic studies. Etoposide inhibits topoisomerase II by preventing the DNA re-joining process, which is part of the enzyme's catalytic cycle. The drug-stabilized enzyme-DNA covalent reaction intermediate can be trapped for detection in cellular and *in vitro* assay systems.[2] Analysis of **1** – **5** treated cells for protein-DNA complex formation[12] revealed no significant induction above the background levels detected in cells treated with Me_2SO alone (Table 1). Since cell-based assay used is relatively insensitive and the transport properties of **1** – **5** are unknown, DNA cleavage was also measured *in vitro* with etoposide as the positive control. As shown in Table 1, compounds **1** – **5** were inactive in this assay. The results for **4** and **5** were concentration-independent over a 100-fold range, suggesting that a type of interference observed using DNA-interactive inhibitors was probably not responsible for the lack of activity.[14] Moreover, autoradiographic analysis of reaction products separated on agarose gels did not reveal limited cleavage, which could have remained undetected by the precipitation assay method (Data not shown). Restricted cleavage *in vitro* has been

reported for certain inhibitors.[15] Thus, underlying mechanism(s) of enzyme inhibition by **1** – **5** is still unclear. Based on present findings, **3** – **5** are worthwhile for further evaluation, due to their selectivity for topoisomerase II and the absence of a cleavable DNA-protein complex. This class of inhibitors is considered to be useful for antitumor drug development.[16]

Table 1. Evaluation of **1** – **7** as DNA Topoisomerase Inhibitors

Compound	% Inhibition of DNA topoisomerase I activity (50 μM)	% Inhibition of DNA topoisomerase II activity (50 μM)	% Cellular protein-DNA complex formation[b] (50 μM)	*% In vitro* topoisomerase II-DNA complex formation[c] (50 μM)
1	100	100	—[a]	—[a]
2	100	100	—	—
3	—[a]	100	—	—
4	—	100	—	—[d]
5	—	100	—	—[d]
6	100	100	N.T.[e]	N.T.[e]
7	100	100	N.T.[e]	N.T.[e]
Etoposide	—	100	100	100

[a] "—"; No activity.
[b] ^{3}H-cpm in potassium-SDS insoluble materials were; 54 ± 1, mock-treated control; 50 ± 20, treatments with **1** – **5** and 964 ± 131 for etoposide.
[c] ^{32}P-cpm in potassium-SDS insoluble materials were; 114 ± 13, DNA/enzyme controls; 93 ± 35, treatment with **1** – **5** and 857 ± 30 for etoposide.
[d] Compounds were evaluated at 1, 10, 50 and 100 μM.
[e] Not tested.

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10. **Compound 4** : A white powder (from H_2O); mp 199–203 °C; $[\alpha]_D^{18}$ –185.9° (*c*=0.49, MeOH); ^{1}H-NMR (acetone-d_6+D_2O) δ 1.80–2.30 (8H, m, 2xquinic acid H_2-2 and 6), 3.66, 3.77 (each 1H, dd, *J*=3.0, 9.0 Hz, quinic acid H-4), 3.96 (1H, d, *J*=2.5 Hz, H-2), 4.18 (2H, m, 2xquinic acid H-5), 4.51 (1H, d, *J*=2.5 Hz, H-1), 5.21, 5.31 (each 1H, m, quinic acid H-3), 6.41 (1H, dd, *J*=2.0, 8.0 Hz, H-6'), 6.49 (1H, d, *J*=2.0 Hz, H-2'), 6.65 (1H, s, H-8), 6.67 (1H, d, *J*=8.0 Hz, H-5'), 6.94 (1H, s, H-5), 7.63 (1H, s, H-4).
Compound 5 : A white powder (from H_2O); mp 182–185°C; $[\alpha]_D^{18}$ –168.7° (*c*=0.29, MeOH); ^{1}H-NMR (acetone-d_6+D_2O) δ 1.82–2.26 (8H, m, 2xquinic acid H_2-2 and 6), 3.60–3.75 (2H in total, m, quinic acid H-4), 3.91, 3.93 (1H in total, each d, *J*=2.5 Hz, H-2), 4.09–4.19 (2H in total, m, 2xquinic acid H-5), 4.47, 4.52 (1H in total, each d, *J*=2.5 Hz, H-1), 5.18, 5.27 (each 1H, m, quinic acid H-3), 6.40, 6.42 (1H in total, dd, *J*=2.0, 8.0 Hz, H-6'), 6.56 (1H, d, *J*=2.0 Hz, H-2'), 6.64 (1H, s, H-8), 6.66, 6.68 (1H in total, each d, *J*=8.0 Hz, H-5'), 6.93 (1H, s, H-5), 7.60, 7.62 (1H in total, each s, H-4). The ^{1}H-NMR data showed the presence of conformational isomers.
11. *DNA topoisomerase assays* : Etoposide was from the Natural Products Laboratory, School of Pharmacy, (UNC-CH). All compounds were dissolved in Me_2SO at a concentration of 20 mM and diluted immediately prior to use with H_2O. Me_2SO in reactions was 0.25% (v/v). Calf thymus enzymes purchased from TopoGen (Columbus, OH) were assayed acording to the suppliers instructions using plasmid pRYG (200 ng/reaction) and P4 DNA (150 ng/reaction) as substrates for topoisomerase I and II, respectively. Preparation of substrates and analysis of reactions were by standard procedures described previously.[5,6]
12. *Potassium-SDS precipitation assay for protein-DNA complexes* : The effect of compounds on the formation of protein-associated DNA breaks was evaluated using the potassium-SDS assay method. The procedures, adapted from the method of Rowe *et al.*[13] for use in cultured KB cells and *in vitro* reactions with purified enzymes, have been detailed in our previous work.[5,6] All treatments were done in duplicate. Cleavage reactions *in vitro* were also analyzed using agarose gel electophoresis and autoradiography in order to examine whether a restricted pattern of cleavage was induced by treatments with **1** – **5**.
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