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THE SYNTHESIS OF 4-DESOXY-2-AZAPODOPHYLLOTOXINS

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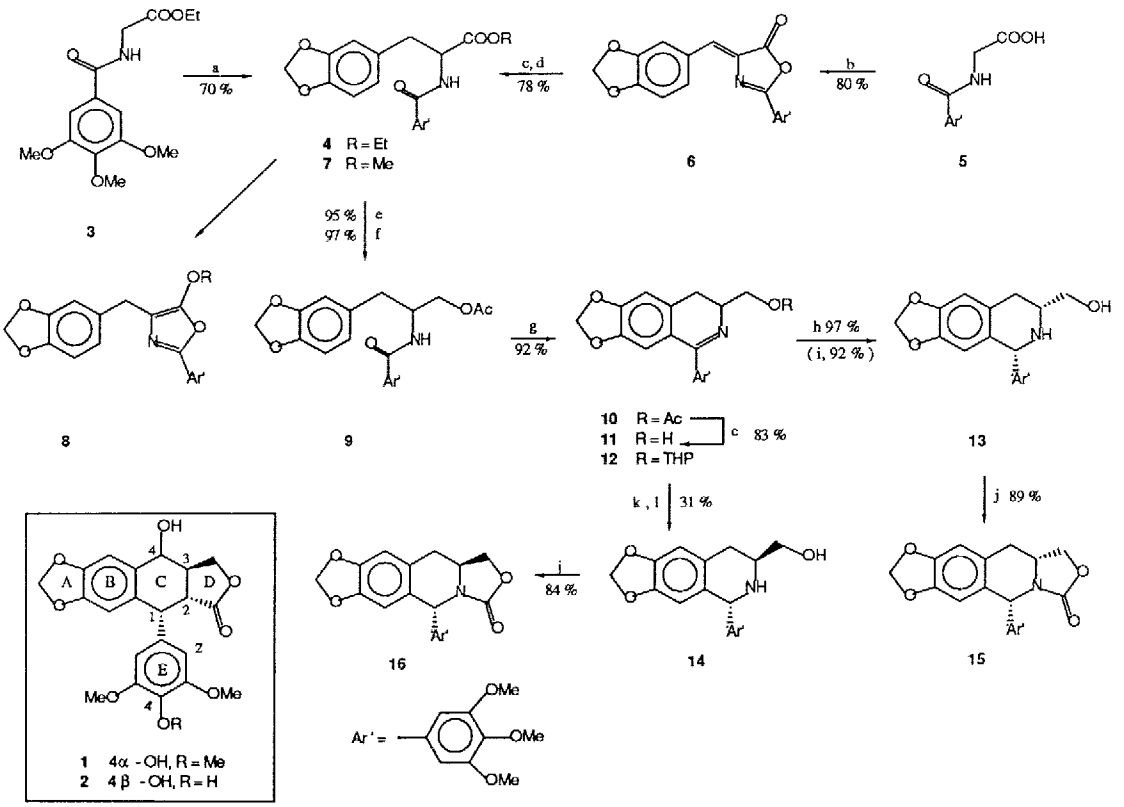
Abstract : 4-Desoxy-2-azapodophyllotoxins, tetrahydroisoquinoline analogues of podophyllotoxin, have been synthesized and evaluated for their anti-tumor activities.

Already some time ago we have embarked on a study of the synthesis and physiological activity of a number of tetrahydroisoquinoline analogues of podophyllotoxin **1**[1,2]. A recent publication in this area[3] prompts us to disclose part of our work.

Podophyllotoxin (**1**), a cytotoxic constituent of the plant species Podophyllum peltatum[4] has attracted considerable research activities which culminated in the synthesis of two derivatives of 4'-demethyl-epipodophyllotoxin **2**, teniposide[5] and etoposide[5] currently in use as chemotherapeutic agents in several human tumor treatments[6]. Although in the last decade the total synthesis of podophyllotoxin has found a renewed[7] interest since Gensler's synthesis in 1966[8], we thought it would be worthwhile to develop methods towards the more readily accessible 2-aza analogues and to evaluate their antitumor activity. In this letter we describe the synthesis and some properties of 4-desoxy-2-azapodophyllotoxin analogues.

In a first approach, we selected the Bischler-Napieralski method[9] for the construction of the intermediate dihydroisoquinoline **11** (scheme 1). The hippuric ester **3** was alkylated with piperonyl bromide to the acyclic precursor **4**[10]. Alternatively the methyl ester **7** was obtained via reaction of the acid **5** with piperonal yielding oxazolon **6**[11]. Subsequent methanolysis and catalytic hydrogenation yielded **7**. Although **4** and **7** are substrates for the projected cyclization step, the expected dihydroisoquinolines were not formed. Under several reaction conditions, only the alkoxy-oxazoles **8** were obtained in ca 40 % yield[12]. Apparently this failure to produce the desired product is due to the presence of the ester function. Therefore the reaction sequence was altered : reduction of the ester function in **4** or **7** and protection of the alcohol as the acetate afforded **9** as a new candidate for the Bischler-Napieralski reaction. As expected, **9** cyclized in high yield to the dihydroisoquinoline **10**. Methanolysis of the acetate and aluminum hydride reduction, efficiently afforded the 1,3-cis-isomer **13** as the sole product. The same isomer was obtained upon catalytic hydrogenation. On the other hand aluminum hydride reduction of the corresponding THP ether **12** led in 93 % yield to a 2:1 mixture of the cis and trans isomers **13** and **14**, which, after removal of the protecting group, could be separated by chromatography. A more efficient method for obtaining 1,3-trans-isomers is given in scheme 2 (vide infra). Finally in both isomers **13** and **14** ring D was formed which led to the 4-desoxy-2-azapodophyllotoxins **15** (m.p. 229°C) and **16** (m.p. 186°C). The relative configurations of both isomers were determined using NOE-difference ^{1}H NMR spectroscopy : irradiation of H_1 led only in the cis-isomer **15** to a response of the H_3-signal.



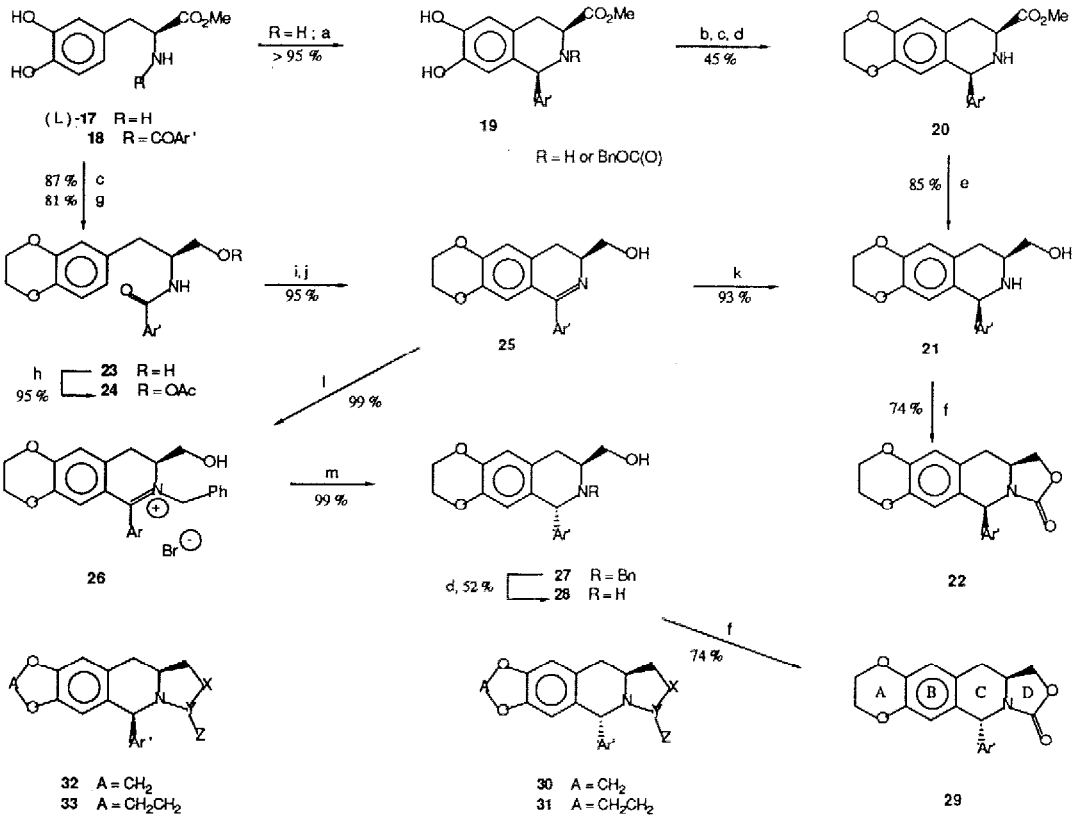
a) LDA (2 eq), TMEDA (2 eq), THF, –78°C, 90 min then $ArCH_2Br$ (1 eq), THF, –78°C - rt, 4 h; b) ArCHO, Ac_2O, NaOAc; c) K_2CO_3, MeOH, rt; d) H_2 (1 atm), Pd/C; e) $LiBH_4$, DME, Δ, 2 1/2 h; f) Ac_2O, Py, rt; g) PCl_5 (3 eq), CH_2Cl_2, rt, 30 min, then $AlCl_3$ (2 eq); rt, 18 h; h) AlH_3, THF, –30°C - rt, 90 min; i) HClg, acetone; H_2 (1 atm), Pt/C, EtOH, MeOH (1:2), rt then $NaHCO_3$; j) $COCl_2$, NEt_3, CH_2Cl_2, O°C; k) AlH_3.THF (2 eq), Δ, 2 h, l) 2 N HCl, THF, rt.

Scheme 1

Screening of the antitumor activity of these molecules and their analogues in the antitubuline test[13,14] and in a number of human tumor cell lines revealed *inter alia* the importance of an ethylenedioxy substitution pattern (ring A) on the aromatic ring B in the 2-azapodophyllotoxin skeleton. The synthesis of such optically active analogues starting respectively from methyl esters of L– and D–DOPA is described in scheme 2 for the L-isomer **17**.

Since it has been shown by Kametani et al.[15], that the Pictet-Spengler reaction with aromatic aldehydes is only possible on a benzene nucleus with a p-hydroxy group, advantage of the nature of the starting material was taken. The reaction gave the cis-isomer **19** as the sole product. Subsequent formation of the ethylenedioxy ring necessitated prior protection of the amine as the benzyl carbamate; the 3–step sequence of **19** to **20** was carried out in 45 % unoptimized overall yield. Finally reduction to **21** and ring D formation gave the analogue **22** ($[\alpha]_D^{20}$ = –58.8, c = 1.02, MeOH; m.p. 180-3°C). For the alternative Bischler-Napieralski approach the ester function in **18** was selectively reduced to **23** after formation of the ethylene dioxy unit (ring A). However, under the cyclization conditions described for the synthesis of **11** (scheme 1) complete racemization occurred. This problem was circumvented using $POCl_3$ in dry acetonitrile, yielding optically pure dihydroisoquinoline **25**. Methanolysis and

subsequent reduction again afforded only the cis isomer **21**. For obtaining the trans isomer **28** a more efficient method (compare **12** to **15** in scheme 1) was investigated[16]. Quaternization of **25** led to **26** which upon sodium borohydride reduction gave the tertiary amine. Hydrogenolysis of **27**, caused mainly cleavage of the N–benzyl substituent in preference to the doubly benzylic 1,2–bond. A 9:1 ratio of both isomers **28** and **21** was observed, indicating a good stereoselectivity of the $NaBH_4$ reduction step.



a) 3,4,5-trimethoxy-benzaldehyde (1 eq), HOAc, NaOAc, rt, 18 h; b) BnOCOCl (1 eq), NEt_3 (1 eq), CH_2Cl_2, 0°C - rt, 18 h; c) $BrCH_2CH_2Br$ (2 eq), K_2CO_3 (3 eq), Me_2CO, Δ, 24 h; d) H_2, 10 % Pd/C, MeOH, 5 h; e) $LiAlH_4$, THF, rt, 3 h; f) $COCl_2$, NEt_3, CH_2Cl_2, 0°C, 1 h; g) $LiBH_4$ (5 eq), DME, rt, 10 h; h) Ac_2O, Py, rt, 30 min; i) $POCl_3$, MeCN, Δ, 18 h; j) K_2CO_3, MeOH, rt, 20 min; k) AlH_3, THF, –30°C - rt, 2 h; l) BnBr, MeCN, Δ, 48 h; m) $NaBH_4$, MeOH, –70°C, 1 h.

Scheme 2

Finally **28** was converted into the trans 2–azapodophyllotoxin analogue **29** ($[\alpha]_D^{25}$ = -115.8, c = 0.57; CH_2Cl_2; m.p. 247-8°C). In the same way, starting from D–DOPA, *ent* **22** ($[\alpha]_D^{20}$ = +62, c = 1.00; MeOH; m.p. 191-3°C) and *ent* **29** ($[\alpha]_D^{25}$ = +117.9, c = 0.36; CH_2Cl_2; m.p. 252-5°C) were obtained.

Next to the above described 4-desoxy-2-azapodophyllotoxin analogues containing the 2-oxotetrahydro-1,3-oxazole ring, also the 2-oxotetrahydro-1,3-imidazole and other heterocyclic D ring analogues have been prepared in order to evaluate their antitumor properties. Treatment of **13**, **14**, **21** and **28** or their amino-analogues with thiophosgene, thionyl chloride or (thio)phosphoryl chloride afforded **30**, **31**, **32** and **33** respectively (X = O, NH

or NMe and Y-Z = C=S, S-O, P(S)Cl or P(O)Cl. Ring closure with thionyl chloride or with phosphoryl chloride gave two isomers which could be separated by column chromatography. Precursors for D-rings, containing two nitrogen atoms, were prepared from N-BOC protected **13** and **14** via Swern oxidation and reductive amination or were formed by $LiAlH_4$ reduction of the amide obtained from ester **20**.

The antitumor activity of all analogues has been screened in-vitro in several human tumor cell-lines. The most active compounds have X = O or N and Y-Z identical to C=O or S-O. In addition, for the optically active compounds (scheme 2), good activities were found for a 1,3-cis, 3R-configuration (*ent* **22**, *ent* **32** and *ent* **33**) or a 1,3-trans, 3S-configuration (**29**, **30** and **31**). Details of the physiological activity will be published elsewhere.

The synthesis and evaluation of the antitumor activity of other analogues differing in aromatic substitution (ring B and E) and the nature of the heterocyclic D ring are in progress.

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References and Notes

1. Vandewalle, M.; Van der Eycken, J.; Van Wijngaarden, I.; Van Hes, R.; Hulkenberg, A.; Keet, C.; Patent appl. A 87 C 73.149 EP 251.361; JP 87.283.960 and Annual Drug Data report **1988**, 10, 510.
2. Hulkenberg, A.; Van Hes, R.; Vandewalle, M.; Van der Eycken, J.; Van Wijngaarden, I.; Xth Int. Symp. Med. Chem., Aug 15-19, Budapest **1988**, 26; Annual Drug Data Report **1988**, *10*, 850.
3. Pearce, H.L.; Bach, N.J.; Cramer, T.L.; Tetrahedron Lett.; **1989**, *30*(8), 907; no reference to ref. 1 and 2 is given.
4. Hartwell, J.L.; Schrecker, A.W.; Fortschr. Chem. Org. Naturst., **1958**, *15*, 83.
5. Keller-Juslén, C.; Kuhn, M.; von Wartburg, A.; Stähelin, H.; J. Med. Chem., **1971**, *14*(10), 936.
6. Jardine, I.; Med. Chem., **1980**, *16*, 319
7. Van der Eycken, J.; De Clercq, P.; Vandewalle, M.; Tetrahedron, **1986**, *42*(15), 4285 and 4297; Andrews, R.C.; Teague, S.J.; Meyers, A.I.; J. Am. Chem. Soc., **1988**, *110*, 7854 and references cited in these publications.
8. Gensler, W.J.; Gatsonis, C.D.; J. Org. Chem., **1966**, *31*, 4004.
9. Fodor, G.; Nagubandi, S.; Tetrahedron, **1980**, *36*, 1279; Fodor, G.; Gal, J.; Phillips, B.A.; Angew. Chem., **1972**, *84*, 947.
10. Krapcho, A.P.; Dundulis, E.A.; Tetrahedron Lett., **1976**, 2205.
11. Baltazzi; Q. Rev., Chem. Soc., **1955**, *9*, 150.
12. Reeve, W.; Paré, P.J.; J. Am. Chem. Soc., **1957**, *79*, 675. During a final literature screening for the patent application, this paper was found in which the preferred formation of **8** was indicated in the title. Interestingly the authors mentioned that their original intention was aimed at 2-azapodophyllotoxin analogues !
13. Gaskin, F.; Cantor, C.R.; Shelanski, M.L.; J. Mol. Biol., **1974**, *89*, 737.
14. Zavala, F.; Guénard, D.; J. Med. Chem., **1980**, *23*, 546.
15. Kametani, T.; Fukumoto, K., Kigasawa, K., Hiiragi, M., Ishimaru, H.; Heterocycles, **1975**, *3*(4), 311.
16. Ishida, A.; Nakamura, T.; Irie, K.; Oh-Ishi, T.; Chem. Pharm. Bull., **1985**, *33*(8), 3237.

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