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STUDIES ON THE MACROCYCLIC PART OF THE TRICHOTHECENE SATRATOXIN: PARTIAL SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP

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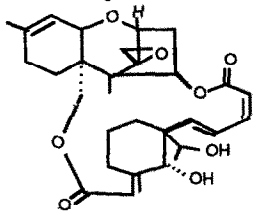
Abstract: Analogs of the macrocyclic diester part of satratoxin were synthesized, which could be considered as precursors of the natural compound. A preliminary study of the structure-activity relationship and of the specificity of these compounds is described.

Satratoxin **1** is a toxic metabolite of *Stachybotrys atra* isolated from foods contaminated with the fungus[1]. As a representant of the trichothecene series, it has generated a great deal of interest owing to the wide spectrum of biological activity exhibited by this class of compounds[2]. Notably antileukemic activity (P388) was established for some of these mycotoxins[3].



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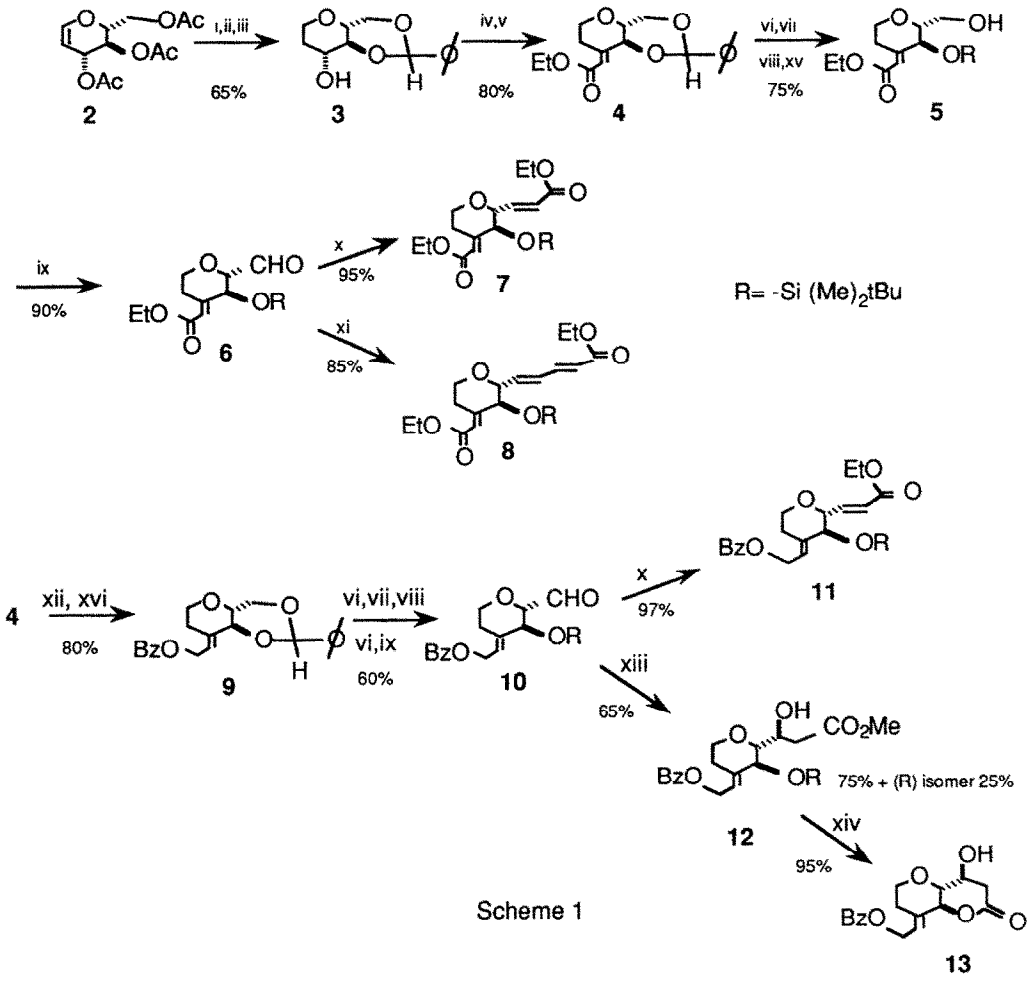
Although large scale fermentation is a classical and convenient source for such compounds[4], it precludes the study of precursors and analogs of the natural products. Furthermore it is not known whether the macrocyclic moiety of these compounds has biological activity by itself or has an enhancement effect when coupled with the trichothecene skeleton, since these molecules are generally much more active than the parent trichothecene.

With this question in mind we have studied a synthetic route to this type of compounds and we describe here the synthesis and biological evaluation of some of the possible precursors of the macrocyclic part of satratoxin. A retrosynthetic analysis showed us that glucal would be a convenient starting material[5].

The synthesis is outlined in scheme 1. Commercially available tri-O-acetyl-D-glucal was hydrogenated, deacetylated and finally protected as the benzylidene derivative **3**. Compound **3** was oxidized with chromium reagents in the presence of molecular sieves[6], then reacted with triethyl phosphonoacetate in alcaline medium to give the unsaturated ester **4**. The orientation of the double bond was ascertained by n.m.r. experiments (N.O.E.). A four steps sequence of protection-deprotection gave **5**, which was smoothly oxidized with Swern reagent[7] to the aldehyde **6**. Finally aldehyde **6** was reacted with either triphenyl phosphoranylidene acetate to give **7** or triphenyl phosphoranylidene crotonate to give **8**.



Scheme 1

Scheme 1- (i): H_2, Pd/C, MeOH, 50°C, 3h, 60 psi; (ii): MeONa (0.1 eq), MeOH, rt, 1h; (iii): $PhCH(OMe)_2$, DMF, p-toluene sulfonic acid (0.01eq), rt, 2h; (iv): PDC, CH_2Cl_2, 4A molecular sieves, rt, 2h; (v): $(EtO)_3P(O)CH_2CO_2Et$, THF, KOH (1.7eq), 0°C, 0.5h; (vi): CF_3CO_2H (90%), rt, 0.5h; (vii): $(Ph)_3CCl$, pyridine, rt, 10h; (viii): $tBu(Me)_2SiCl$, DMF, Imidazole, rt, 10h; (ix): DMSO (2eq), $(COCl)_2$ (1.2eq), CH_2Cl_2, -70°C, 1h; (x): $Ph_3P{=}CH\text{-}CO_2Et$, CH_2Cl_2, 0°C, 0.3h (xi): $Ph_3P{=}CH\text{-}CH{=}CH\text{-}CO_2Et$, CH_2Cl_2, 0°C, 2.5h; (xii): DIBAH (1.2eq), THF, 0°C, 0.25h; (xiii): $BrCH_2CO_2Et$ (5eq), Zn (1.1eq), ether, 0°C, sonication, 0.75h; (xiv): CF_3CO_2H (90%, EtOH), 0°C, 0.5h; (xv): HCO_2H (90%, ether), rt, 0.2h; (xvi): pyridine (5eq), PhCOCl (1.1eq), CH_2Cl_2, rt, 0.2h.

Similarly, monoester compounds were obtained via DIBAH reduction of **4**, and subsequent benzoylation to give **9**, which was further transformed into aldehyde **10** using the same reactions sequence as for **6**.The aldehyde **10** was then reacted with triphenyl phosphoranylidene acetate to give **11**, or with ethyl bromoacetate in a Reformatsky reaction to give **12**. It is worth to note that this latter reaction gave 75% of a major isomer. The configuration of this isomer was determined by homonuclear decoupling n.m.r. experiments on the lactonised derivative **13**. The coupling observed between the proton at the carbon of undetermined configuration and the vicinal proton, was consistant with a trans diaxial relationship, thus the (S) configuration could be securely assigned for this compound[8].

The cytotoxicity of compounds **7, 8, 11, 12** and **13** has been tested. The biological evaluations were performed on mice spleen lymphocytes stimulated by PHA (Phaseolus Vulgaris Phytohemagglutinin) for either 48 h (corresponding to fully DNA synthesis) or 72 h (return to steady quiescent state) and malignant human lymphocytes : Daudi lymphoma cells. The rate of cellular multiplication was evaluated by tritiated thymidine incorporation measurement[9] which gives valuable estimation of DNA synthesis. The results were expressed as the concentration that inhibits cell growth by half (IC 50). The results are summarised in the table:

Table 1: IC-50 in 10^{-9} mole/ml culture

compound	Daudi	Lympho-PHA 48h	Lympho-PHA 72h
7	9.3 ± 0.74	8.8 ± 0.6	17.6 ± 3.2 *
8	2.2 ± 0.37	0.73 ± 0.17 *	1.1 ± 0.26
11	0.6 ± 0.06	0.7 ± 0.08	1.4 ± 0.16 **
12	10 ± 0.65	20 ± 0.97 **	20 ± 2.0 **
13	3.5 ± 0.17	8.8 ± 1.08 **	13 ± 1.6 **

Each experiment was made in triplicate. Results ± sm. The cytotoxicity of each compound for the different cells was performed in the same experiment. The comparison between the Daudi cells and the stimulated lymphocytes was made by the DUNNETT test according to ANNOVA computerized program. * P= 0.05; ** P= 0.01.

Compound **8** which is the closest to the natural diester in satratoxin, exhibited a high toxicity for normal cells. A greater selectivity for dividing cells was found with all the other compounds.

Although the observed activities were much less important than for the overall macrocyclic compound (for instance the structurally related myrotoxin was toxic to lymphocytes at a concentration of 10^{-12} mol/ml)[10], these results should be taken into account in the global activity of the macrocyclic trichothecene, since the diester linkage is likely to be hydrolysed in the biological medium. Furthermore it is known that verrucarol, the trichothecene portion of these macrocycles was 10^5 times less active than the macrocyclic compounds[11]. This is the result of the synergic effect of the macrocyclic part of these compounds. These preliminary results are encouraging enough to pursue the synthesis and studies of other analogs.

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5- Although we were aware that D-glucal would give the enantiomer of our target compound, our choice was driven by practical reasons for a model synthesis.

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8- Physical constants of all the compounds described are in agreement with the assigned structures. The 1H N.m.r. data (CDCl3, 300MHz) for typical compounds are as follows: 7: 0.15, 0.2(2s, 6H), 0.9(s, 9H), 1.25, 1.3(2t, 6H), 2.14(ddd, 1H), 3.39(ddd, 1H), 3.72(ddd, 1H), 3.79(d, 1H), 3.91(d, 1H), 4.03-4.23(m, 5H), 5.99(s, 1H), 6.03(dd, 1H), 7.0(dd, 1H). **8**: 0.1, 0.18(2s, 6H), 0.8(s, 9H), 1.23(t, 3H), 1.3(t, 3H), 2.17(m, 1H), 3.36(dd, 1H), 3.6-3.8(m, 2H), 3.9(m, 1H), 4.02-4.16(m, 5H), 5.85(d, 1H), 5.97(s, 1H), 6.09(dd, 1H), 6.36(dd, 1H), 7.2(dd, 1H). **11**: 0.1, 0.15(2s, 6H), 0.95(s, 9H), 1.2(t, 3H), 2.2(m, 1H), 2.76(d, 1H), 3.37(dd, 1H), 3.64-3.83(m, 2H), 4.10-4.25(m, 3H), 4.89(d, 2H), 5.86(dd, 1H), 6.06(d, 1H), 7.05(dd, 1H), 7.38-8.04(m, 5H). **12**: 0.05, 0.1(2s, 6H), 0.88(s, 9H), 1.18(t, 3H), 2.13(m, 1H), 2.39(dd, 1H), 2.51-2.71(m, 2H), 2.88(d, 1H), 3.27(dd, 1H), 4.0-4.17(m, 4H), 4.28(dd, 1H), 4.83(d, 2H), 5.82(dd, 1H), 7.33-7.96(m, 5H). **13**: 2.31(m, 1H), 2.64(dd, 1H), 2.88(d, 1H), 3.12-3.28(m, 2H), 3.53(dd, 1H), 4.09-4.23(m, 2H), 4.32(d, 1H), 4.83-5.04(m, 2H), 6.02(dd, 1H), 7.4-8.07(m, 5H).

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