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APOTRICHOTHECENE REARRANGEMENT IN MACROCYCLIC TRICHOTHECENE DERIVATIVES [1]

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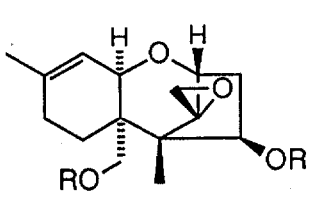
ABSTRACT:The apotrichothecene rearrangement was carried out with verrucarin A (**1**), J (**2**) and roridin A (**3**). The synthesis of the macrocyclic diester **9** of verrucarol (**4**) is described. The immunosuppressive (MLR) and cytotoxic (P-815) activities of the compounds **6-9** were determined *in vitro*.

The trichothecenes belong to a class of sesquiterpenoid natural products which are produced by moulds, especially various species of *Fungi imperfecti*. Many members of the family display a wide range of biological activities [3]. The rearrangement of the trichothecene skeleton during treatment with mineral acids is well known and leads by initial cleavage of the oxirane ring to the apotrichothecene skeleton which is biological inactive [4,5,6]. In connection with our programme directed towards the synthesis of unnatural macrocyclic trichothecene derivatives [7] we investigated the apotrichothecene rearrangement of the macrocyclic trichothecenes verrucarin A (**1**), J (**2**)and roridin A (**3**). Our interest was focused on the question whether this rearrangement generally takes place without touching the macrocyclic moiety of the mycotoxins and on the biological activity of such rearranged macrocyclic products which to our knowledge had not yet been examined.

The trichothecenes **1**,**2** and **3** were treated with H_2SO_4 in dioxane. In contrast to the simple trichothecene verrucarol (**4**) [4] the rearrangement of the macrocyclic derivatives proceeded at higher temperature (80°, 4-6h) and led to the products **6**,**7** and **8** with intact macrocyclic segments. The analogous rearrangement of **1** with HCl in CH_2Cl_2 was reported by *Grove* [6] and led to the chloroapotrichothecene **5**. The structures of the products obtained were deduced from the MS and NMR data.

In order to examine the relations between the substitution pattern of the macrocyclic segment and the biological activity, the simple 18-membered macrocyclic trichothecene **9** was synthesized using dodecanedioic acid as building block for the macrocyclic part. Verrucarol (**4**) was esterfied with the mixed dianhydride of dodecanedioic acid and pivalic acid leading to the macrocyclic diester **9** in about 30% yield.

Biological Activity: The cytotoxic and immunosuppressive activities of the new compounds were determined by *P.Hiestand, Sandoz AG Basel*,using the P-815 [8] and MLR [9] *in vitro* tests. The rearranged compounds **6 - 8** showed a weak immunosuppressive (MLR) activity (IC_{50} values in µg/ml: **6**: 0.07, **7**: 0.05, **8**: 0.05) On the other hand **6 - 8** were found to be inactive in the cytotoxicity (P-815) test. (IC_{50} values for **6 - 8**: > 1µg/ml). The macrocyclic analogue **9** was found to be moderately active (MLR: 0.2 µg/ml, P-815: 0.088 µg/ml). It's activity was shown to be about hundred times lower than that of natural macrocyclic compounds, a fact which demonstrates the necessity of a suitably functionalized macrocyclic segment to obtain a high biologically active compound.



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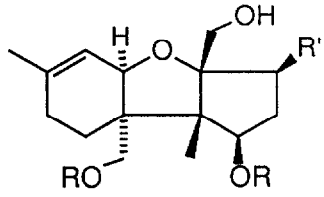
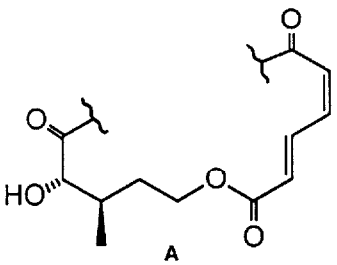
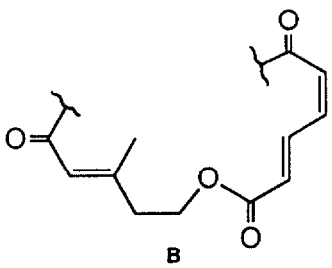
2 R = B
3 R = C



5 R = A, R' = Cl
6 R = A, R' = OH
7 R = B, R' = OH
8 R = C, R' = OH



A



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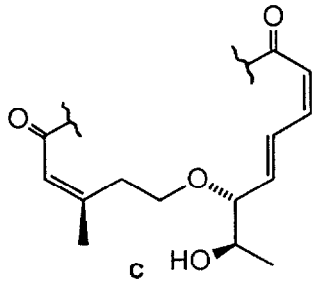

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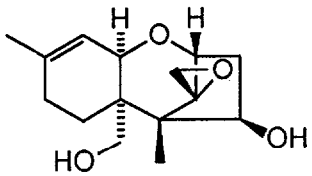

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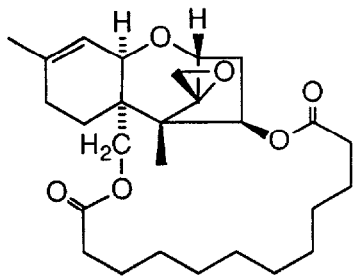

9

Experimental Procedures: Apotrichothecene Rearrangement: A soln. of 54 mg of verrucarin A (**1**) in 2.5 ml of dioxane and 1.5 ml of 1N H_2SO_4 was heated to 80°. After 6h the dioxane was distilled off and the aqueous layer extracted with CH_2Cl_2. Column chromatography (SiO_2, CH_2Cl_2 / MeOH 96:4) afforded 23 mg (41%) of **6.** (The same procedure yielded 59% of **7** and 73% of **8**, respectively.)

Characterization of **6**: m.p.: 230-300°: dec. (from CH_2Cl_2 / hexane).$[\alpha]_D^{23}$= +117°. EI-MS: 520 (M^+, 10.0%), 265 (6.2%), 248 (4.7%), 189 (7.2%), 175 (12.7%), 163 (43.0%), 145 (13.5%), 125 (21.1%), 105 (100%), 85 (57.1%), 55 (31.6%). ^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si): 8.46 (*ddd*, *J* = 1, 11.5, 15.5, H-C(4")); 6.77 (*dd*, *J* = 11.0, 11.5, H-C(3")); 6.10 (*dd*, *J* = 1, 11.0, H-C(2")); 6.06 (*d*, *J* = 15.5, H-C(5"); 5.91 (*d*, *J* = 6.5, H-C(4)); 5.51(*ddd*, *J* = 1.5, 1.5, 5.0, H-C(10)); 4.57 (*m*, H-C(5')); 4.56 (*d*, *J* = 12.5, H-C(15)); 4.43 (*dd*, *J* = 2.5, 6.0, with D_2O: *d*, *J*=2.5, H-C(2')); 4.28 (*dd*, *J* = 5.0, 5.0, with D_2O: *d*, *J* = 5); 4.20 (*br.d*, *J* = 12.5, H-C(15)); 4.05 (*dt*, J = 3.0, 11.5, H-C(5')); 3.96 (*dd*, *J* = 8.5, 12.0, with D_2O: *d*, *J* = 12.0, H-C(13)); 3.80 (*dd*, *J* = 4.5, 12.0, with D_2O: *d*, *J* = 12.0, H-C(13)); 3.67 (*d*, *J* = 5.0, H-C(11)); 2.61 (*d*, *J* = 6.0, exchangeable with D_2O, OH); 2.50 (*m*, H-C(3')); 2.35 (*d*, *J* = 6.0, exchangeable with D_2O, OH); 2.21 (*m*, exchangeable with D_2O, OH); 2.19 (*ddd*, *J* = 5.0, 6.5, 15.5, H-C(3)); 2.05 (*d*, *J* = 15.5, H-C(3)); ca.1.93 (*m*, H-C(4')); ca. 1.75 (*m*, H-C(4')); 1.74 (*br.s*, H_3C(16)); 1.03 (*s*, H_3C(14)); 0.90 (*d*, *J* = 7.0, H_3C(6')). ^{13}C-NMR (101 MHz, $CDCl_3$, Me_4Si): 174.9, 165.4, 164.8, 140.4, 140.2, 139.4, 127.4, 124.8, 118.0, 96.1, 80.1, 79.4, 75.3, 73.6, 64.6, 63.4, 61.9, 59.9, 47.9, 39.6, 33.2, 32.2, 27.5, 23.3, 22.7, 13.0, 11.9.

Characterization of **7**: m.p. 242- >300°: dec. (from CH_2Cl_2 / hexane).$[\alpha]_D^{23}$= -26° EI-MS: 502 (M^+, 5.8%), 265 (5.5%), 248 (6.3%), 217 (16.5%), 189 (6.8%), 175 (13.1%), 163 (43.4%), 145 (16.3%), 125 (23.6%), 113 (100%), 95 (99.0%), 80 (54.8%), 67 (56.0%), 52 (59.5%). ^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si): 8.40 (*ddd*, *J* = 1, 11.5, 16.0, H-C(4")); 6.68 (*ddd*, *J* = 1, 11.5,11.5, H-C(3")); 6.03 (*dd*, *J* = 1, 11.0, H-C(2")); 6.00 (*d*, *J* = 16.0, H-C(5")); 5.85 (*m*, H-C(4)); 5.83 (*s*, with D_2O: *d*, *J* = 1, H-C(2')); 5.52 (*m*, H-C(10)); 4.56 (*ddd*, *J* = 5.0,5.0,11.5, H-C(5')); 4.48 (*d*, *J* = 12.5, H-C(15)); 4.27 (*m*, with D_2O: *dd*, *J* = 3.0, 5.0, H-C(2)); 4.09 (*ddd*, *J* = 6.5,6.5,11.5, H-C(5')); 3.94 (*dd*, *J* = 9.0,11.5 with D_2O: *d*,*J* = 11.5, H-C(13)); 3.84 (*dd*, *J* = 4.0, 11.5, with D_2O: *d*,*J* = 11.5, H-C(13)); ca. 3.82 (H-C(11)); 3.77 (*d*, *J* = 12.5, H-C(15)); ca.2.52 (exchangeable with D_2O, OH); ca.2.32 (exchangeable with D_2O, OH); 2.25 (*d*, *J* = 1.5, H_3C(6')); 1.72 (*br.s*, H_3C(16)); 1.06 (*s*, H_3C(14)).

Characterization of **8**: m.p. 175-178° (from CH_2Cl_2 / ether). $[\alpha]_D^{23}$= +78°. EI-MS: 550 (M^+, 0.7%),249 (21.0%), 212 (43.0%), 163 (17.2%), 131 (12.1%), 105 (48.1%), 85 (100%). CI-MS: 568 ($[M+NH_4]^+$) (46.4%), 284 (100%). FAB-MS: 551 ($[M+H]^+$, 14.0%), 154 (100%). ^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si): 7.78 (*dd*, *J* = 11.5, 15.5, H-C(4")); 6.69 (*dd*, *J* = 11.5, 11.5, H-C(3")); 6.04 (*dd*, *J* = 3.5, 15.5, H-C(5")); 5.73 (*d*, *J* = 11.0, H-C(2")); 5.84 (*dd*, *J* = 2.0, 6.0, H-C(4)); 5.51 (*m*, H-C(10)); 4.39 (*d*, *J* = 12.5, H-C(15)); 4.23 (*m*, with D_2O: *dd*, *J* = 2.5, 5.0, H-C(2)); 4.16 (*d*, *J* = 12.5, H-C(15)); 3.95 (*br.d*, *J* = 11.5: with D_2O sharp *d*, *J* = 12.0)); 3.81 (*br.d*, *J* = 11.5, with D_2O: sharp *d*, *J* = 12.0, H-C(13)); 3.72 (*br.d*, *J* = 5.0, H-C(11)); ca.3.64 (*m*, H-C(6")); ca. 3.62 (*m*, H-C(7")); 3.55 (*m*, H_2C(15)); 2.77 (*br.s*, exchangeable with D_2O, OH); 2.68 (*d*, *J* = 6.0, exchangeable with D_2O, OH); 2.52 (*br.s*, exchangeable with D_2O, OH); ca. 2.30 (*br.s*, exchangeable with D_2O, OH); ca.2.28 (*m*, H-C(3')); ca. 2.27 (*m*, H-C(3)); ca. 1.98 (*m*, H-C(3)); 1.73 (*br.s*, H_3C(16)); 1.19 (*d*, *J* = 6.0, H_3C(8")); 1.08 (*s*, H_3C(14)); 1.02 (*d*, *J* = 6.5, H_3C(6')). ^{13}C-NMR (400 MHz, $CDCl_3$,

Me_4Si): 174.6, 164.9, 144.6, 140.2, 139.9, 127.3, 118.1, 117.2, 95.3, 84.4, 79.4, 77.8, 75.1, 74.4, 70.7, 69.3, 64.5, 63.5, 59.4, 47.5, 39.2, 35.2, 34.1, 27.5, 23.3, 22.5, 18.2, 14.4, 13.0.

Preparation of **9**: To a soln. of 149 µl of Et_3N, 49 mg of dodecanedioic acid and 47 µl of pivalic acid in 10 ml of CH_2Cl_2, 50 mg of dimethylaminopyridine and 70 ml of CH_2Cl_2 were added. The soln. was stirred for 30 min., then a soln. of 47 mg of verrucarol (**4**) in 1 ml of CH_2Cl_2 was added dropwise and stirred for 20h. The solvent was evaporated and the residue purified by column chromatography (SiO_2, ether / petroleum ether (7:3) which yielded 29 mg (30%) of **9**. M.p. 122.4-124.2° (from hexane). EI-MS: 460 (M^+, 11.9%), 417 (3.4%), 247 (22.0%), 230 (23.6%), 217 (36.4%), 202 (9.8%), 187 (10.0%), 173 (11.8%), 159 (12.2%), 145 (8.7%), 121 (30.1%), 105 (100%), 91 (31.0%), 81 (49.9%), 69 (28.9%), 55 (66.8%). ^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si): 5.55 (*dd*, *J* = 4.5, 8.0, H-C(4)); 5.42 (*br.d*, *J* = 5.0, H-C(10)); 4.10 (*d*, *J* = 12.5, H-C(15)); 4.06 (*d*, *J* = 12.5, H-C(15)); 3.82 (*d*, *J* = 5.0, H-C(2)); 3.64 (*br.d*, *J* = 5, H-C(11)); 3.14 (*d*, *J* = 4.0, H-C(13)); 2.84 (*d*, *J* = 4.0, H-C(13)); 2.56 (*dd*, *J* = 7.5, 15.5, H-C(3)); ca. 1.98 (*m*, H-C(3)); 1.71 (*br.s*, H_3C(16)); 0.88 (*s*, H_3C(14)). ^{13}C-NMR (101 MHz, $CDCl_3$, Me_4Si): 173.5, 173.4, 141.1, 118.3, 79.1, 75.3, 67.0, 65.3, 63.2, 48.7, 47.9, 43.4, 37.1, 34.4, 34.3, 28.1, 28.0, 27.7, 27.2, 27.0, 27.0, 26.9, 24.6, 24.5, 23.2, 20.8, 7.6.

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