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SPIROEPOXYCYCLOHEXADIENONE DIMERS: CYTOTOXIC BISALKYLATING AGENTS

J.-P. Gesson* and M. Mondon
Laboratoire de Chimie 12, Associé au CNRS
40, Avenue du Recteur Pineau, F-86022 Poitiers

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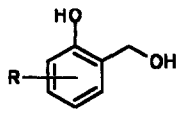
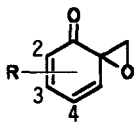
Abstract: Spiroepoxycyclohexadienone dimers **C** obtained by Adler oxidation of salicyl alcohols display cytotoxicity against L1210, HT29 and A549. The alkylating properties of **1** are shown by ready addition of benzylamine to generate a new azabicyclo[2,2,2]octane ring system, and of N-methylbenzylamine to give mono- (together with partial aromatization) and bis-alkylated products.

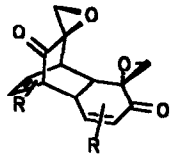
The oxidation of salicyl alcohols **A** by sodium periodate has been first reported by Adler in 1971.[1] The initially formed spiroepoxycyclohexadienones **B** rapidly dimerize, except, for example, in the presence of bulky substituents (such as tertiobutyl) at C-2 or C-4[2] or of a methoxy group at C-3,[3] by way of a regio and stereospecific Diels-Alder cycloaddition to give dimers **C**. The presence in both **B** and **C** of different electrophilic centers (epoxide, enone) suggests that these compounds may well behave as alkylating agents toward biomolecules (thiols, amines), and thus may display cytotoxicity or mutagenicity. Although the reactivity of spiroepoxycyclohexadienones **B** bearing bulky substituents (vide supra) toward several nucleophiles has been reported already by Reiss,[4] no study has been made on the dimers **C**. Reiss has shown that the sensitive[5] compounds **B** react with nitrogen nucleophiles to give mainly **D** resulting from initial nucleophilic attack on the less substituted carbon atom of the epoxide with cleavage of a C-C bond and rearomatization.



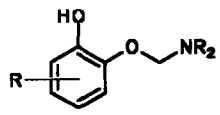
A



B



C

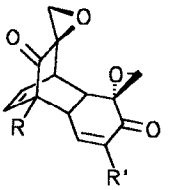


D

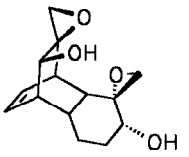
As part of a general study on the chemistry and biological properties of compounds such as **B** and **C**, we report here the high cytotoxicity of some dimers **C** and the behavior of the simplest one **1** toward model primary and secondary amines.

Dimer **1** is obtained in 88% isolated yield by treatment of 2-hydroxybenzyl alcohol with 1.1 eq. of $NaIO_4$ in 4-1 water-EtOH. Similarly oxidation of 2-hydroxy-3-methoxybenzyl alcohol affords **2** (52%). In order to prepare mixed dimers resulting from cycloaddition between two different dienones **B**, the co-oxidation of the two former salicyl alcohols (1/1 ratio) has been carried out. In this case, dimers **1** and **2** are isolated in 32 % yield together with **3** (15%).[6] The alternative mixed dimer **4** is not isolated, but the possible Cope rearrangement which will interconvert **3** and **4** should be taken in consideration.[7]

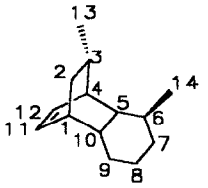
The $NaBH_4$ reduction of **1** has also been carried out in order to test the possible activity of the corresponding diol(s). The major diol **5** (38%) results from initial 1,4-addition of hydride to the enone moiety followed by stereoselective reduction of both ketones (**6** is also isolated in 29% yield). In both cases, hydride approach syn to the oxygen epoxide is prevented for steric reasons: at C-7 because **1** is an endo adduct and at C-2 because of 1,3-diaxial interaction with H-10.[8]



1 R=R'=H
2 R=R'=OCH_3
3 R=H, R'=OCH_3
4 R=OCH_3, R'=H



5
6 (Δ8,9)



All compounds synthesized were tested for their cytotoxicity on three different cell lines (L1210 leukemia, HT29 colon cancer and A549 lung adenocarcinoma) using a proliferation assay (MTT reduction).[9]

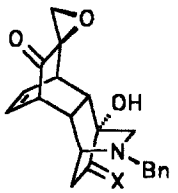
Results are reported in Table 1 together with those of the known anticancer agent doxorubicin (DOX) in the same test. Dimer **1** exhibits a potent cytotoxicity on all three cell lines, the dimethoxy derivative **2** displaying a much lower activity except on A549. Surprisingly the monomethoxy derivative **3** is almost inactive on L1210 and A549. Diols **5** and **6** are also inactive.

Table I: IC_{50} (μM) in the MTT-assay.

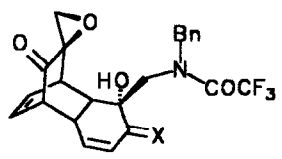
	L1210	HT29	A549
DOX	0.04	0.12	0.10
1	0.18	0.3	0.75
2	1.0	3.6	1.2
3	>36	5.8	17.5
5	19.5	6	18
6	22.5	17	31.5
8	>28	27	>28

As suggested earlier, the potential alkylating properties of dimers **C** may explain their cytotoxicity. Thus the reactivity of **1** with simple amines such as benzylamine and N-methylbenzylamine has been studied under mild conditions (CH_2Cl_2, 20°C).

Treating **1** with benzylamine affords two compounds, **7** (49%)[6] and **8** (9%), arising from a double addition of the nucleophile on the β carbon of the enone moiety (C-9) and at C-14, the less hindered carbon of one of the epoxide functions, to generate a new azabicyclo[2,2,2]octane ring system.



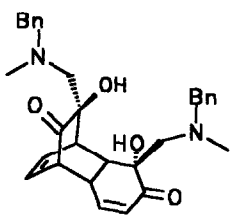
7 X=O
8 X=NBn



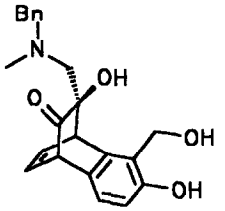
9 X=O
10 X=NBn

Their structures are consistent with ^{1}H and ^{13}C NMR spectra and particularly, for **7**, by the observation of H-14 at δ 2.56 and 3.03 ppm (2H, 2d, J= 10.5 Hz), of C-6 at δ 75.9 ppm (instead of δ 57.6 ppm in **1**) and of C-9 at δ 54.8 ppm. Although the C-6 tertiary alcohol could be acetylated without incident (Ac_2O, Pyridine, cat. DMAP), exposure of **7** and **8** to $(CF_3CO)_2O$ in pyridine results in ring cleavage to give respectively **9** and **10** thus confirming the structure assignments.

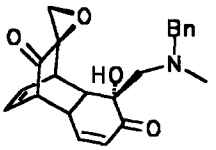
The reaction of **1** with N-methylbenzylamine appears to be more complex leading, for example in one experiment, to **11** (52%), **12**(17.5%) and **13** (16.5%).[6] However, the outcome of this reaction is highly variable, since the isomeric **14**[6] is sometimes the only monoalkylated product isolated or a mixture of **13** and **14** may be obtained (the amount of the aromatized compound **12** is also variable). The site of alkylation is easily determined by ^{13}C NMR: for compounds **11** and **14** C-2 appears at δ 210 (δ 203 ppm for **1**), C-3 appears at δ 70-73 ppm for **11**, **12** and **14** and 58.4 ppm for **13** (δ 57.5 or 57.7 ppm for **1**), C-6 appears at δ 78 ppm for **11** and 69.9 ppm for **13** (δ 57.5 or 57.7 ppm for **1**). Structure of **12** is consistent with the formation upon acetylation (Ac_2O, pyridine, cat. DMAP) of a mixture of di- and triacetates which both have a characteristic Ar-CH_2-OAc signal at δ 5.17 ppm.



11



12



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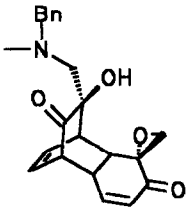

14

The results obtained with these two model amines show that no Michael-type addition is detected with the secondary amine (the reversibility of the addition may explain this behavior) but both epoxides are cleaved by this nucleophile while surprisingly only one reacts with benzylamine. The interesting bis-alkylated derivative **7** obtained from the latter has been tested in the MTT-assay and shows as expected inactivity on all three cell lines. Thus the high potency of **1** and the reduced activity of the methoxy derivatives **2** and **3** (the inactivity of **3**, compared to **2**, against L1210 is surprising in vue of the similarity between these two compounds) suggest that a bis-alkylation may be the cytotoxic event and thus the search of active analogs may be guided by this principle.

In conclusion, the spiroepoxycyclohexadienone dimers **C** are interesting cytotoxic compounds, their ease of synthesis (including monochiral compounds[10]) and structural manipulation suggest further studies to determine their potential use as antitumor agents.

Acknowledgements: Financial support from CNRS and Laboratoires HOECHST is deeply acknowledged. We thank Dr. Sedlacek (Behringwerke, Marburg, Germany) for cytotoxicity tests.

References and Notes:

1. Adler, E.; Brasen, S.; Miyake, H. *Acta Chem. Scand.* **1971**, *25*, 2055-2069.
2. Becker, H.-D.; Bremholt, T.; Adler, E. *Tetrahedron Lett.* **1972**, 4205-4208.
   Becker, H.-D.; Bremholt, T. *Tetrahedron Lett.* **1973**, 197-200.

3. Corey, E.J.; Dittami, J.P. *J.Am.Chem.Soc.* **1985**, *107*, 256-257.

4. Cacioli, P.; Reiss, J.A. *Aust. J. Chem.* **1984**, *37*, 2525-2535.

5. The spiroepoxydienones are light and heat sensitive and easily give salicylaldehydes together with complex products on storage at room temperature.

6. All new compounds have been characterized by spectral (^1H and ^{13}C NMR, MS and IR) and analytical (HRMS or microanalysis) data. Representative ^1H (200 MHz) and ^{13}C (50.13 MHz) NMR data of key compounds are given below (δ in ppm (mult., J in Hz), TMS as internal standard, $CDCl_3$), using a Bruker WP200SY spectrometer.

3: δ 2.74 (1H, dd, 9.2 and 1.8), 2.90 (m, 4H), 3.17 (1H, d, 6.1), 3.50 (1H, d, 7), 3.63 (s, 3H), 5.55 (1H, d, 4.8), 6.15 (1H, t, 7) and 6.64 (1H, t, 7) ppm. **7:** δ 2.43 and 2.75 (2H, 2dd, 19.5 and 2.2), 2.56 and 3.03 (2H, 2d, 10.5), 2.87 and 3.16 (2H, 2d, 6), 2.93 (1H, m), 3.32 (1H, t, 3), 3.80 (2H, s) and 6.18 (2H, m) ppm. ^{13}C δ 38.55, 39.4, 39.05, 43.9, 52.7, 54.8, 57.4, 58.9, 59.6, 75.9, 127.4, 128.4, 128.5, 129.2, 133.4, 137.8, 202.0 and 212.0 ppm. **11:** δ 2.19 and 2.23 (6H, 2s), 2.41 and 2.62 (2H, 2d, 13.5), 2.62 and 2.93 (2H, 2d, 13.5), 3.20 (m, 4H), 3.32 and 3.58 (2H, 2d, 13), 3.54 and 3.73 (2H, 2d, 12.9), 5.80 (1H, t, 7.2), 5.98 (1H, d, 10.2), 6.18 (2H, m), and 7.30 (10H, s) ppm. ^{13}C: δ 39.8, 39.9, 43.7, 44.0, 44.4, 52.9, 62.7, 63.5, 63.6, 68.0, 73.1, 77.95, 126.8, 127.3, 127.7, 127.9, 128.3, 128.9, 129.1, 135.0, 137.4, 138.9, 145.0, 201.0 and 210.0 ppm. **12:** δ 2.41 (3H, s), 2.54 and 2.68 (2H, 2d, 13.9), 3.55 and 3.87 (2H, 2d, 12.9), 4.04 (1H, d, 6), 4.28 (1H, d, 6), 4.78 (2H, ABq, 12.5), 6.37 (1H, t, 6), 6.43 (1H, t, 8), 6.54 (1H, t, 6), 6.93 (1H, d, 8) and 7.34 (5H, s) ppm. ^{13}C: δ 38.95, 39.7, 42.3, 53.3, 44.5, 53.8, 58.4, 63.85, 67.65, 78.1, 127.0, 128.2, 128.8, 128.9, 129, 134, 139, 145, 200 and 204 ppm. **13:** δ 2.24 (s, 3H), 2.60 and 2.78 (2H, 2d, 10.8), 2.92 and 3.12 (2H, 2d, 6.2), 2.97 (1H, dd, 8.4 and 1.8), 3.05 (2H, m), 3.36 and 3.52 (2H, 2d, 12.8), 5.93 (1H, ddd, 8, 6.5 and 1.4), 6.01 (1H, dd, 10.1 and 1.4), 6.26 (1H, dd, 10.1 and 3.9), 6.38 (1H, ddd, 8.6 and 1.4) and 7.30 (5H, s) ppm. ^{13}C: δ 44.2, 47.6, 57.2, 57.5, 62.8, 63.4, 69.9, 114.5, 123.5, 125.1, 127.4, 127.5, 128.4, 129.3, 131.3, 134.0, 138.2, 138.8, 155.0 and 204.0 ppm. **14:** δ 2.36 (3H, s), 2.47 and 2.69 (2H, 2d, 13.7), 2.90 (2H, ABq, 6.6), 3.08 (1H, d, 7.5), 3.33 (1H, d, 6), 3.50 (1H, m), 3.57 and 3.78 (2H, 2d, 12.7), 5.97 (1H, dd, 7.5 and 6), 6.18 (1H, dd, 10.2 and 1.4), 6.41 (1H, t, 7.5), 6.62 (1H, dd, 10.2 and 2.4) and 7.3 (5H, s) ppm. ^{13}C: δ 36, 39.6, 43.9, 46.9, 52.9, 58, 58.5, 62.4, 63.6, 72.4, 127.6, 128.1, 128.4, 129.1, 131, 134.9, 137.9, 147.1, 193 and 210 ppm.

7. Unpublished results. For Cope rearrangements in similar ring systems see: Ansell, M.F.; Gosden, A.F.; Leslie, W.J. *Tetrahedron Lett.* **1967**, 4537.

8. This is confirmed for **5** by the observation of nOe's between H-2 (δ 3.57 ppm, d, J= 2.9 Hz) and H-11 (δ 6.15 ppm, m, overlapping H-12), and H-2 and H-13 (δ 2.86 ppm, d, J= 4.6 Hz).

9. Exponentially growing L1210, A549 or HT29 tumor cells at a density of 5.10^3/mL in RPMI were incubated in a 96 well microtiter plate for 72 hrs (37°C, 5% CO_2, 95% rel. humidity) with various concentrations of each test substance. Control consisted of cells exposed to fresh medium only. Quadruplicate wells were prepared for each drug concentration and for control. After 65 hours, 50 μL of a solution of MTT (2.5 mg/mL) in PBS were added. The MTT will be reduced by viable cells to a red insoluble formazan dye. After additionnal 7 to 24 hours incubation (depending on the cell used) the supernatant medium was carefully removed. The formazan dye was solubilized by adding 100 μL DMSO to each well followed by gentle shaking. The extinction was mesured for each well using a Multiscan photometer 340 CC, Fa. Flow, at 492 nm. Results were expressed as the ration of the extinction after incubation with test substances over that of control. The coefficient of variation for replicate experiments was less than 15%.

10. Yamashita, D.S.; Rocco, V.P.; Danishefsky, S.J. *Tetrahedron Lett.* **1991**, *32*, 6667-6670.