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KETO C-GLYCOSIDES. A NEW CLASS OF ANTITUMOR COMPOUNDS.

Jean Herscovici*§, M. Idriss Bennani-Baiti†, Charles Frayssinet† and Kostas Antonakis§.
§Laboratoire de Chimie Organique Biologique et Spectroscopique. Institut de Recherches Scientifiques Sur le Cancer, CNRS, 94801 Villejuif, France.
†Laboratoire de Pathologie Cellulaire. Institut de Recherches Scientifiques Sur le Cancer, CNRS, 94801 Villejuif, France.

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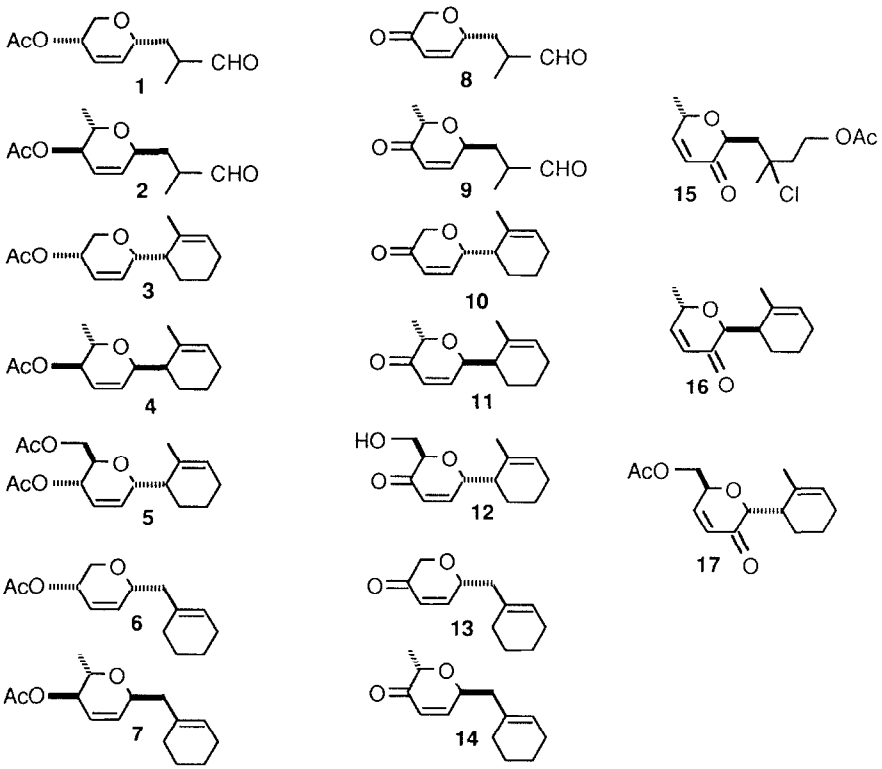
Abstract. A series of 4-keto unsaturated C-glycosides was synthesized from 0-acetyl C-glycosides. These compounds and some parent 2-keto unsaturated C-glycosides were tested for cytotoxic activity against $LFCl_2A$ cells (Rat hepatocarcinoma cells). C-Glycosides with an acyclic aglycone have not been found cyctotoxic, whereas 1-methyl cyclohexenyl and methylene cyclohexenyl C-glycosides possessed IC_{50} values of 9-0.03 μM with **11** being the most potent .

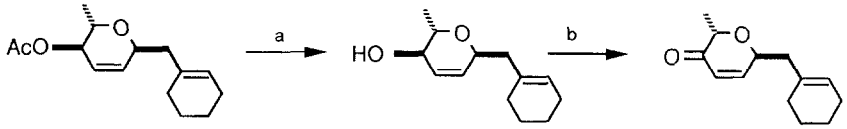
Screening of plant extracts has led to the isolation of a large number of sesquiterpene derivatives having cytotoxic properties[1,2]. The activity of these compounds derives from the presence of a C=C-C=O system such as α-methylene γ-lactones, αβ-unsaturated ketones, lactones, esters or epoxides[3] which act as alkylating agents by a Michael addition with biological nucleophiles[1,2,4]. However it is widely recognized that the usefulness of most natural sesquiterpene lactones has been limited by their relatively high toxicity[5]. Investigation to reduce toxicity has resulted in synthetic modification of α-methylene lactones and preparation and testing of synthetic analogues[6-10].

One rational of designing less toxic drugs is to introduce an alkylating group to other classes of biologically active molecules. Thus our group has previously reported that keto or unsaturated keto nucleosides exhibited cytotoxic properties[11] and inhibited tumor growth *in vivo*[12].

Recently we have reported new methodologies to prepared 2,3 unsaturated C-glycosides with a polyfunctional aglycone by the condensation of an olefin with a peracetylated glycal[13-15]. The significant antitumor activity demonstrated by keto unsaturated nucleosides and related derivatives encouraged us to further synthesize and evaluate keto unsaturated C-glycosides. Because of the wide range of structures available it would be reasonable to assume that these molecules could be valuable tools for a better understanding the structure activity relationships in order to design antitumor agents acting with a better selectivity.

To explore the effect of the aglycone on cytotoxic activity, 4-keto unsaturated C-glycosides **8** and **9** with a linear aglycone and enones **10-14** with a 1-methyl cyclohexene or a methylene cyclohexene unit bond to the anomeric carbon, were prepared. The synthesis was carried out in two steps (scheme 1) from the previously reported acetates **1-7** prepared by methods developed in our laboratory[13-15] Deacetylation





a MeONa MeOH RT b PDC 4Å MS or MnO_2

scheme 1

using sodium methoxide then oxidation with PDC/4Å MS[16] afforded the enone in 60-80% yield However under these conditions the D-gluco derivative **12** was isolated in 20% only. Treatment of **12** with 10 equivalents of MnO_2 gave the β-keto alcohol **12** in 50 % yield.

The cytotoxicity of the unsaturated keto C-glycosides was determined against LFCl2A cells, an established cell line derived from an hepatocarcinoma induced in the Commentry rat by dimethylamino azobenzene (DAB)[17-19]. In addition some 2-keto unsaturated C-glycosides[14] were also evaluated. All the result was summarized in Table 1.

Table 1 *in vitro* cytotoxicity

Compounds	IC_{50} (μM)
7	40
8	60
9	80
10	3
11	3×10^{-2}
12	7
13	9×10^{-2}
14	9
15	40
16	3×10^{-1}
17	3
5-fluoro uracil	28.5

Examination of the data deserves further comments. C-glycosides **8**, **9** and **15** with a linear aglycone showed no activity. On the other hand C-glycosides **10-14** and **16-17** were found to be cyctotoxic with IC_{50} values ranging from 9 to 0.03 μM. As expected the antitumor activity was dependant on the presence of the keto group as evidenced by the results recorded for acetate **7**.

In addition Table 1 revealed clearly the importance of the substitution at C-5. Thus, 5-hydoxymethyl or pentahexopyranosyl C-glycosides were moderately cytotoxic whereas 6-deoxy keto unsaturated C-glycosides **11**(IC_{50} 0.03 μM), **14** (IC_{50} 0.09 μM) and **16** (IC_{50} 0.3 μM) were the most active compounds. Finally the 4-keto derivatives seemed to be slightly more cytotoxic than the 2-keto C-glycosides.

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References and Notes.

1. S. M. Kupchan, M. A. Eakin, A. M. Thomas, *J. Med.Chem.*, **1971,***14*, 1147.
2. K-H. Lee, E-S. Huang, C. Piantadosi, J. A. Pagano, T. A. Geissman. *Cancer Res.* **1971**, *31*, 1649.
3. J. M. Cassady and M. Suffness. in Anticancer Agents Based on Natural Products Models. M. J. Cassady and J. D. Douros. Academic Press 1980.
4. I. H. Hall, K.-H. Lee, E. Mar, C. O. Starnes. *J. Med. Chem.*, **1977**, *20*, 333.
5. G. A. Howie, P. E. Manni, J. M. Cassady. *J Med. Chem.*, **1974**, *17* , 840..
6. S. S. Dehal, B. A. Marples, R. J. Stretton, J. R. Traynor. *J. Med Chem.*, **1980**, *23*, 90.
7. K.-H. Lee, E.-C. Mar, M. Okamoto, I. H. Hall. *J. Med. Chem.*, **1978**, *21*, 819.
8. P. A. Grieco, J. A. Noguez, Y. Masaki, K. Hiroi, M. J. Nishizawa. *J. Med. Chem.*, **1977**, *20*, 71.
9. P. J. Stang, W. L. Treptow. *J. Med. Chem.*, **1981**, *24*, 468.
10. K.-H. Lee, G. K. Rice, I. H. Hall, V. Amarnath *J. Med. Chem.*, **1987**, *30*, 568.
11. K. Antonakis, *Advances in Carbohydrates Chemistry and Biochemistry;* Academic Press. **1984,** *42*, 227.
12. M. Alaoui-Jemali, C. Lasnes, K. Antonakis,I. Chouroulinkov. *Mutagenesis*, **1986,** *1*, 411.
13. J. Herscovici, K. Muleka, K. Antonakis, *Tetrahedron. Lett.*, **1984**, *25*, 5653.
14. J. Herscovici, S. Delatre, K. Antonakis, *J. Org. Chem.*, **1987**, *49*, 5691.
15. J. Herscovici, L. Boumaïza, K. Muleka, K. Antonakis, *J. Chem. Soc. Perkin 1*, **1990**, 1995.
16. J. Herscovici,.M.J. Egron, K. Antonakis.*J. Chem.Soc., Perkin I.* **1982**, 1967.
17. C. Lafarge-Frayssinet, M. Garcette, R. Emanoil-Ravier, E. Morel-Chany, C. Frayssinet .in "Liver cells and drugs" A. Guillouzo Ed. Colloque INSERM, John Libbey Eurotext Ltd **1988**, *164*, 465.
18. R. Cassingena, C. Lafarge-Frayssinet, V. Painchault , S. Estrade, P. Nardeux, C. Frayssinet *Biol Cell.* **1990**, *69*, 113.
19. LFC1$_2$A cells were grown in EMEM (Eagle'sMinimal Essential Medium, Eurobio) supplemented with 10% NCS (New born calf serum, 6 Flow Laboratories), 2 mM glutamine and antibiotics (100 UI penicillin/ml and 100 µg streptomycin/ml). They were usually seeded at 25,000 cells/ml medium in culture flask incubated at 37°C in a humidified incubator with an atmosphere of air/C02 of 95/5 and subcultured twice a week. Cell suspensions (200 µl) were dispensed in wells of microtest plates. The dilutions of keto-C-glycosides to be tested were performed in DMSO, 1 µL of each dilution was added to wells of microplates together with the cells suspensions. Cells were incubated with the cytotoxics for 72 hours. Twelve hours before the cells were harvested, 1 µCi tritiated thymidine was added to each well. Cultures were washed and collected with an automated sample harvester (Skatron) on glass fiber filters (Whatmann). The filters were dried and the radioactivity was counted in omnifluor in a liquid scintillation spectrometer. Each experiment was conducted in triplicate.