Tetrahedron Letters, Vol.31, No.32, pp 4537-4538, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

AGLYCONE FRAGMENTATION ACCOMPANIES ß-GLUCOSIDASE CATALYZED HYDROLYSIS OF SALICORTIN, A NATURALLY-OCCURRING PHENOL GLYCOSIDE

Thomas P. Clausen, John W. Keller, and Paul B. Reichardt*

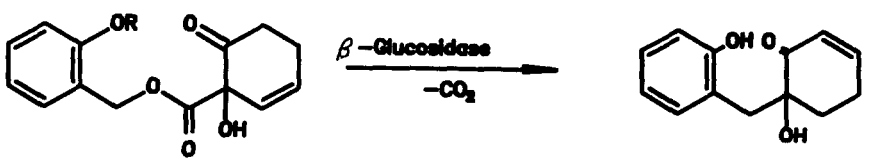
Department of Chemistry, University of Alaska Fairbanks
Fairbanks, Alaska 99775-0520

Summary: Salicortin (**1a**), upon enzyme-mediated glycosidic hydrolysis, undergoes an unusual and rapid fragmentation-recombination reaction leading to **2** via postulated ortho-quinone methide (**3**) and enol (**4**) intermediates.

Phenol glycosides are widely distributed plant secondary metabolites that are generally thought to play an important role in regulating herbivory by insects and mammals.[1] *In vitro*, glucosidase treatment of phenol glycosides provides a mild and efficient method for releasing intact aglycones. In contrast, we report here that enzymatic hydrolysis of salicortin (**1a**),[2] a phenol glycoside that is present[3] in many members of the Salicaceae family and that is toxic to some herbivores,[4,5] releases glucose[3] and leads to a unique fragmentation-recombination reaction of the aglycone that probably involves a transient ortho-quinone methide.

Product studies were carried out with substrate levels of β-glucosidase (1 mg mL^{-1} enzyme,[6] 5mM **1a**, pH 5 acetate buffer, 37 °C). Under these conditions **1a** was consumed, carbon dioxide was produced (75% yield by respirometry), and one major product (**2**)[7] was formed (53% yield by GC) (Scheme 1). No aglycone (**1b**) was detected.

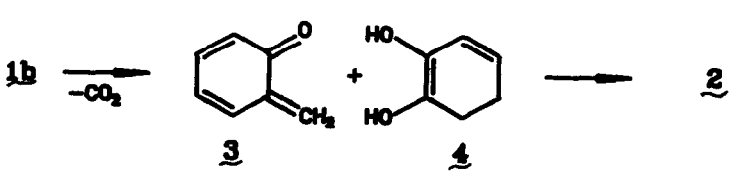
SCHEME 1



1a (R=1-β-D-Glc)
1b (R=H)

2

SCHEME 2



3 **4**

Formation of **2** can be rationalized (Scheme 2) by initial hydrolysis of **1a** to form **1b**. The aglycone (**1b**) then fragments to ortho-quinone methide **3**, carbon dioxide, and an enol (**4**), which subsequently combines with **3** to form **2**. The recombination can be envisioned either as a Michael addition[8] or as a heteronuclear Diels-Alder reaction[9] that forms a hemiketal which subsequently opens to give **2**.

Trace amounts of *o*-hydroxybenzyl alcohol, 6-hydroxycyclohex-2-ene-1-one, and catechol were also identified as reaction products by comparison of the gc-ms of the reaction mixture with authentic compounds. These products may arise from solvent capture, tautomerization, or air oxidation of the fragmentation products, respectively.

β-Glucosidase-catalyzed decomposition of **1a** is accompanied by a slow time-dependent irreversible inhibition of the enzyme. In a typical reaction at pH 6.2, 25 ^{o}C, and 4.4 mM **1a**, the half-life of β-glucosidase activity was 70 min. Enzyme activity could not be restored by repeated ultrafiltration with fresh acetate buffer. The inactivation process, which is still under investigation, may be due to reaction of the β-glucosidase active site with the electrophilic **3**.

The reaction described here is significant for several reasons: 1) it is an unusual enzyme-catalyzed fragmentation-recombination; 2) it represents a new approach to the development of inhibitors of glucosidases for therapeutic purposes and as probes for the glucosidase active site;[10] and 3) it may explain the reported toxicity of certain phenol glycosides[1] and in particular the sensitivity of insects having high endogenous levels of β-glucosidases to dietary **1a**.[4]

Acknowledgements

We thank the National Science Foundation for support of this research (Grant No. BSR 8416461 and BBS-8614587). The mass spectral determination was performed by the Midwest Center for Mass Spectroscopy, an NSF Regional Instrumentation Facility (Grant No. CHE-8620177). Finally, we thank Dr. Larry Byers for his critical review of an earlier version of this manuscript.

References and Notes

(1) Reichardt, P.B.; Clausen, T.P.; Bryant, J.P., *Biological Active Natural Products. Potential use in Agriculture*; Cutler, H.G., Ed.; American Chemical Soc.: Washington D.C., **1988**; pp 130-142.
(2) **1a** was isolated by the method of Clausen, T.P.; Evans, T.P.; Reichardt, P.B. *J. Nat. Prod.* **1989**, *52*, 207.
(3) Pearl, I.A.; Darling, S.F. *Phytochemistry* **1971**, *10*, 3161.
(4) Lindroth, R.L., Scriber, J.M., Hsia, M.T.S. *Ecology* **1987**, *69*, 814.
(5) Bryant, J.P., Clausen, T.P., Reichardt, P.B.; McCarthy, M.C.; Werner, R.A. *Oecologia (Berl.)*. **1987**, *73*, 513.
(6) ICN almond β-glucosidase, 22 units mg^{-1} (1 unit = 1 μmole salicin hydrolyzed min^{-1} at 37^{o}C).
(7) **2**: High resolution mass spectra: found 218.0941; $C_{13}H_{14}O_3$ requires: 218.0943; Infrared ($CHCl_3$): 1690 cm^{-1} (conjugated ketone); ^{1}H-NMR ($CDCl_3$; 90 MHz): δ = 8.5 ppm (1H, broad s, exchangeable with D_2O), 6.7-7.3 (5H, m), 6.2 (1H, ddd, J = 10.0, 2.45, 1.46 Hz), 4.85 (1H, broad s, exchangeable with D_2O), 3.05 (2H, s), 2.45-2.75 (2H, m) and 1.7-2.45 (2H, m). The hydrogen at δ = 6.2 was found to be coupled with hydrogens at δ = 7 and 2.5 by double irradiation experiments. ^{13}C-NMR ($CDCl_3$; 22.5 MHz): δ = 156(s), 151.7(d), 131.8(d), 129.1(d), 125.8(d), 121.9(s), 120.2(d), 117.7(d), 77.5(s), 39.8(t), 33.1(t) and 25.0(t).
(8) Poss, A.J.; Belter, R.K. *Tetrahedron Lett.* **1987**, *28*, 2555.
(9) Marino, J.P.; Dax, S.C. *J. Org. Chem.* **1984**, *49*,3671.
(10) Dale, M.P.; Ensley, H.E.; Kern, K.; Sastry, K.A.R.; Byers, L.D. *Biochemistry* **1985**, *24*, 3530. Fleet, G.W.J. *Chem. in Britain* **1989**, 287. Halazy, S.; Danzin, C.; Ehrhard, A.; Gerhart, F. *J. Am. Chem. Soc.* **1989**, *111*, 3484. Legler, G; Harder, A. *Biochim. Biophys. Acta* **1978**, *524*, 102. Walsh, C.T. *Ann. Rev. Biochem.* **1984**, *53*, 493.

(Received in USA 20 June 1990)