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Epimerization of Annonaceous Acetogenins under Basic Conditions

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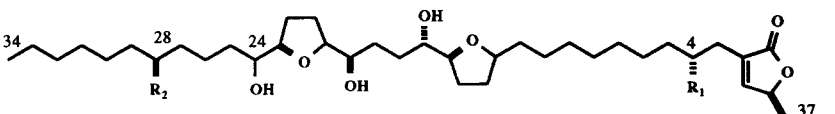
Abstract : In basic conditions, non-4-hydroxylated annonaceous acetogenins may be epimerized by light basic treatment. The two epimers are inseparable by HPLC, have identical spectroscopic data (MS, IR, UV, ^{1}H and ^{13}C NMR). However, specific rotation of the epimeric mixture and enzymatic oxidation after chemical degradation, allow us to characterize this epimerization. In the same reaction conditions, 4-hydroxylated annonaceous acetogenins led to isoacetogenins. © 1997 Elsevier Science Ltd.

A few years ago, we reported that annonaceous acetogenins ended by a saturated α-acetonyl butyrolactone were in fact artifacts of isolation and/or purification due to translactonization of 4-hydroxylated acetogenins[1]. In our continuous efforts toward the isolation and structural determination of acetogenins of Annonaceae, we got some more evidences on the mechanism involved in such a rearrangement, and want to show, in this letter, implications of such event in the described data of known acetogenins. Indeed, during our work on isolation of new acetogenins from *A. atemoya*[2], we treated with Et_2NH a mixture of three C-37 acetogenins composed of two known components (cherimolin-2 or bullatanocin and almunequin or squamostatin-A) and a new one (atemotetronin[2]), in order to isomerize cherimolin-2 the sole 4-hydroxylated acetogenin of this mixture (Figures 1, 2). Indeed, after formation of the translactonized product (isocherimolin-2 or bullatanocinone), the two other products were easily separated by HPLC. Then in order to determine the absolute configuration of the stereogenic center of the butyrolactone of the new compound, we used our recent enzymatic method[3]. Surprisingly we observed that we were in the presence of an epimeric equimolar mixture of *R* and *S* lactic acids, thus indicating a mixture of *R* and *S* C-36 acetogenins.

Figure 1



cherimolin-2 (or bullatanocin) : R^1 = OH, R^2 = H, C-23-C-24 : *threo*
almunequin (or squamostatin-A) : R^1 = H, R^2 = OH, C-23-C-24 : *erythro*

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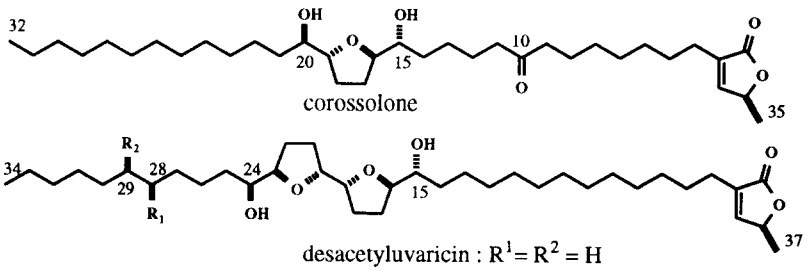
So far about 280 bioactive acetogenins have been isolated and characterized from the Annonaceae family[4], the *S* absolute configuration of the chiral center of the butenolide being usually determined by NMR for the 4-hydroxyacetogenins but only postulated for most of the acetogenins lacking the 4-hydroxyl group. Furthermore, in several cases strong differences in the $[\alpha]_D$ values for a same compound were reported, without satisfaying explanations. For instance corossolone is reported with $[\alpha]_D$ +15[5], +20.7[6], +10[7], all data being recorded in methanol with similar concentrations (c = 0.13, 0.2, 0.18, respectively). Desacetyluvaricin was described with $[\alpha]_D$ +9.3[8] and +19.3[9], squamocin with an $[\alpha]_D$ +0.15[10] and +16.3[11], and squamostatin-A with $[\alpha]_D$ +11[12] and +25[13], again the data were recorded in methanol and with the same concentrations (Figure 2).

Figure 2

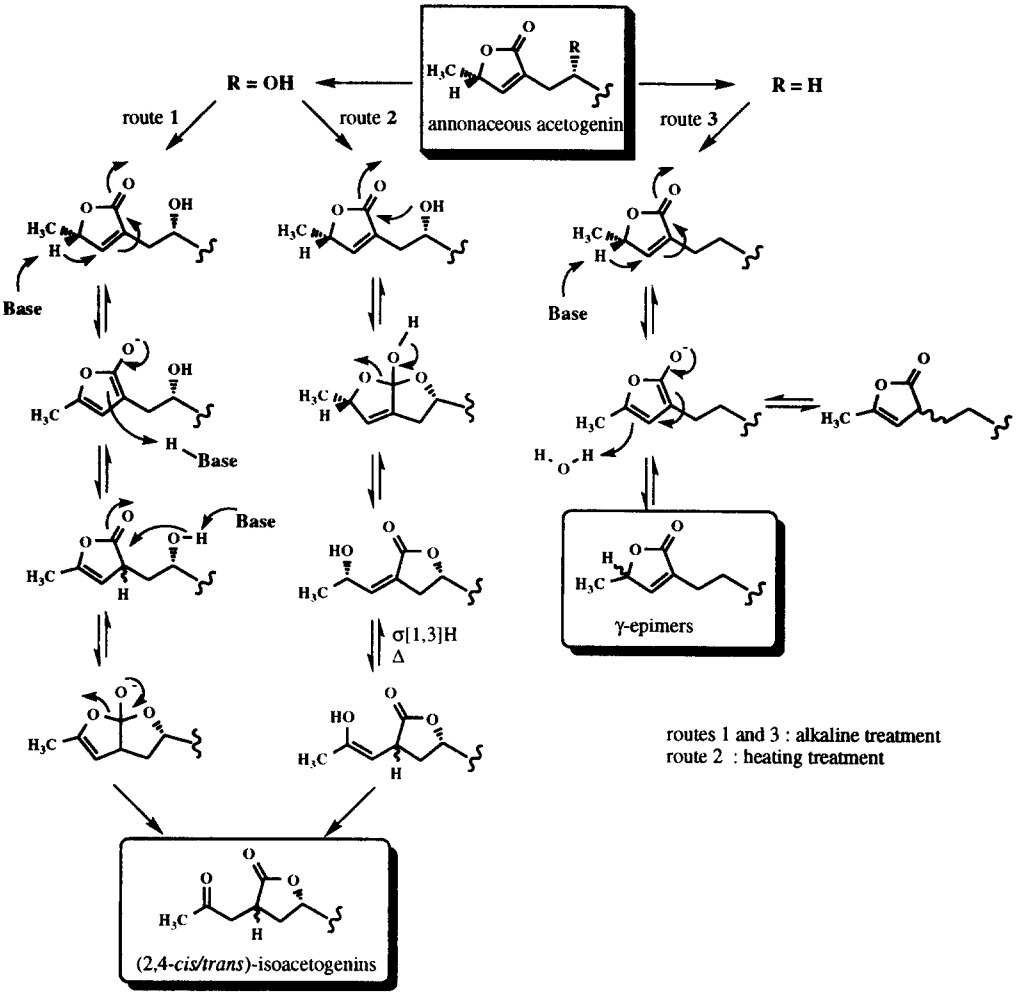


corossolone

desacetyluvaricin : $R^1 = R^2 = H$
squamocin : R^1 = OH, R^2 = H
atemotetrolin : $R^1 = R^2$ = OH

In order to rationalize these findings we choose squamocin (a non C-4 hydroxylated acetogenin) and measured its $[\alpha]_D$ before and after treatment with Et_2NH ($[\alpha]_D$ +24 *vs* +9). Detections of NADH in the L- and D-LDH incubation media of the oxidative mixture, obtained after $RuCl_3$-H_5IO_6 oxidation of squamocin, were performed again before and after basic treatment. We thus observed that γ-epimerization of the butenolide moiety indeed occurred. For the 4-hydroxyacetogenins, when the same light basic treatment was applied we observed the formation of the trans-lactonized products and two mechanisms of translactonization into the corresponding isoacetogenins were proposed[1] (Scheme 1) : route 1, involving hydrogen abstraction at the γ position of the lactone leading to the non-conjugated butenolide intermediate, and route 2 attack of the 4-hydroxyl, leading to the new lactone ring after a σ[1,3] hydrogen migration. At this time it was difficult to either favour or discard one of these two pathways. It is worth noting that translactonization may also occur from the corresponding 4-hydroxyacetogenins by heating in alcohol[1]. However when squamocin was subjected to heating in methanolic solution for 16 hours, no γ-epimerization was detected neither by LDH methodology, nor by $[\alpha]_D$ measurements. This result confirms the occurrence of the first step described in route 2 for the 4-hydroxyacetogenins. The basic γ-epimerization observed with squamocin starts also by a deconjugaison followed by the thermodynamically favoured re-conjugaison (route 3). For the 4-hydroxyacetogenins, the trans-lactonization followed the deconjugaison and finally the keto-enolate equilibrium (which is the driving force of the process) afforded the mixture of iso-acetogenins. We can also conclude that the two mechanisms (routes 1 or 2) could occur according to the experimental conditions : route 1 when basic conditions are applied and route 2 during heating (in route 2, the σ[1,3]H being theoritically reversible, however because of the keto-enol

Scheme 1


R = OH
R = H
route 1
route 2
route 3
annonaceous acetogenin
Base
Base
Base
σ[1,3]H
Δ
γ-epimers
routes 1 and 3 : alkaline treatment
route 2 : heating treatment
(2,4-cis/trans)-isoacetogenins

equilibrium, the saturated γ-lactone is formed). However, for the non-4-hydroxyacetogenins, epimerization of the butyrolactone ring may occur only under light basic conditions.

In conclusion, these findings allow us to state that basic treatment should be avoided, even in the absence of 4-hydroxyacetogenins, in order to exclude γ-epimerization of the terminal butenolide of acetogenins which leads to an epimeric mixture, which is only possible to detect when using our LDH methodology. Moreover, basic γ-epimerization of non 4-hydroxyacetogenins could explain abnormal different published $[\alpha]_D$ values for identical acetogenins. Compilation of these data (concerning the specific rotations of annonaceous acetogenins) allows us to postulate that the sign of the $[\alpha]_D$ of these natural compounds is dependent at the first level on the absolute configuration of the butenolide moiety, and in a less extent on the absolute configurations of the stereogenic centers of the THF core (since a single epimerization of one center of the THF core leads to only a slight change in the value[4]). Finally, only a very few absolute configurations of the stereogenic center of the butenolide part of annonaceous acetogenins has been established without ambiguity[4]. The use of circular dichroism[9] and the "as usual C-34 (36) *S* configuration" sentence should no more be used. Indeed, we strongly recommend to use our enzymatic protocol[3] which allows one to characterize this C-34 or C-36 configuration without errors, even with traces of natural compounds.

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References

1 Duret, P.; Laurens, A.; Hocquemiller, R.; Cortes, D.; Cavé, A., *Heterocycles*, **1994**, *39*, 741-749.

2 Duret, P.; Hocquemiller, R.; Cavé, A., *Phytochemistry*, **1997**, submitted.

3 Duret, P.; Waechter, A.-I.; Figadère, B.; Hocquemiller, R.; Cavé, A.; Piérard, C; Pérès, M., *Tetrahedron Lett.*, **1996**, *37*, 7043-7046.

4 Cavé, A.; Figadère, B.; Laurens, A.; Cortes, D., In *Progress in the Chemistry of Natural Products*; Herz, W., *et al.* Eds.; Springer-Verlag: Wien, New York, 1997, Vol. 70, pp. 81-288.

5 Cortes, D.; Myint, S.H.; Laurens, A.; Hocquemiller, R.; Lebœuf, M.; Cavé, A., *Can. J. Chem.*, **1991**, *69*, 8-11.

6 Yao, Z.-J.; Wu, Y.-L., *J. Org. Chem.*, **1995**, *60*, 1170-1176.

7 Chen, W.-S.; Yao, Z.-J.; Wu, Y.-L., *Youji Huaxue*, **1995**, *15*, 85-88. (C.A., **1995**, *122* : 261023f)

8 Jolad, S.D.; Hoffmann, J.J.; Cole, J.R.; Barry, C.E.; Bates, R.B.; Linz, G.S.; Konig, W.A., *J. Nat. Prod.*, **1985**, *48*, 644-645.

9 Sahai, M.; Singh, S.; Singh, M.; Gupta, Y.K.; Akashi, S.; Yuji, R.; Hirayama, K.; Asaki, H.; Araya, H.; Hara, N.; Eguchi, T.; Kakinuma, K.; Fujimoto, Y., *Chem. Pharm. Bull.*, **1994**, *42*, 1163-1174.

10 Fujimoto, Y.; Eguchi, T.; Kakinuma, K.; Ikekawa, N.; Sahai, M.; Gupta, Y.K., *Chem. Pharm. Bull.*, **1988**, *36*, 4802-4806.

11 Duret, P., Thèse de Doctorat de l'Université Paris-Sud, Châtenay-Malabry, 1997.

12 Fujimoto, Y.; Murasaki, C.; Kakinuma, K.; Eguchi, T.; Ikekawa, N.; Furuya, M.; Hirayama, K.; Ikekawa, T.; Sahai, M.; Gupta, Y.K.; Ray, A.B., *Tetrahedron Lett.*, **1990**, *31*, 535-538.

13 Cortes, D.; Myint, S.H.; Dupont, B.; Davoust, D., *Phytochemistry*, **1993**, *32*, 1475-1482.

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