CHEMILUMINESCENT METHOD IN EVALUATING THE ANTI-OXIDANT ACTIVITIES OF SESQUITERPENE LACTONES

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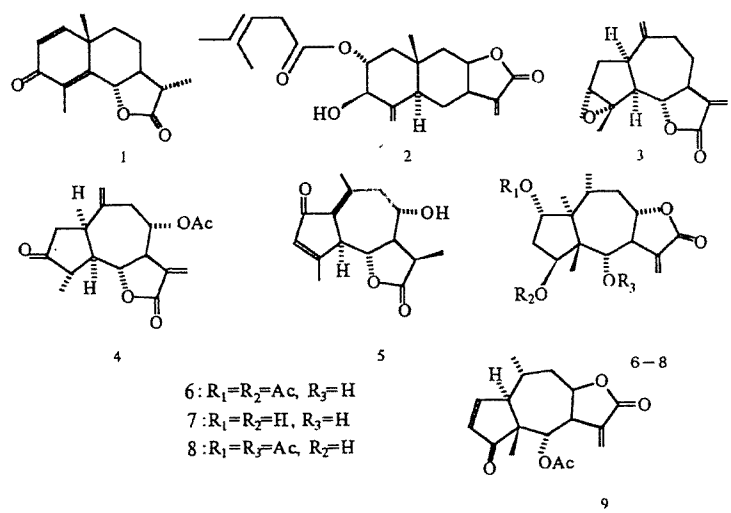
One of the objective and convenient methods of evaluating the antioxidant activities (AOAs) of various systems is the initiation of chemiluminescence (CL) [1]. By this method we have studied *in vitro* the AOAs of nine sesquiterpene lactones isolated from Kazakhstan plants and their modified derivatives (α-santonin (**1**), 3β-hydroxy-2α-senecioyloxyisoalantolactone (**2**), estafiatin (**3**), grosshemin acetate (**4**), grossmizin (**5**), inuchinenolide C (**6**), dideacetylinuchinenolide C (**7**), britanin (**8**), and helenalin acetate (**9**)) [2].

The intensity of the induced CL was determined on a KhLM 1Ts-01 chemiluminometer [3]. As the biological substrata we used a rat brain homogenate. The homogenate was prepared by the careful trituration of the brain in liquid nitrogen, and a 10% solution in phosphate buffer, pH 7.62, was used in the experiment. The CL initiator was 3 mM $FeSO_4 \cdot 7H_2O$. All the compounds were investigated at identical quantitative ratios: initial concentration, 20 mM; final concentration, 0.1 mM.



Analysis of the chemiluminograms showed that the curve of CL initiated by Fe^{2+} ions was similar to that observed for egg yolk lipoproteins [4]. We separated the lactones under investigation into two groups: antioxidants the action of which prolonged the latent period, and poorly effective compounds.

In the first group, the clearest AOA was exhibited by estafiatin (**3**) and helenalin acetate (**4**). While in the control group the latent period τ amounted to 0.01 ± 0.04 min, for estafiatin it was 1.63 ± 0.41 min ($P < 0.05$), and for helenin acetate 1.63 ± 0.17 min ($P < 0.001$). Another common property of these antioxidants in *in vitro* experiments was an increase in the latent period τ of a repeated CL: 1.71 ± 0.38 min ($P < 0.05$) for estafiatin (**3**) and 1.59 ± 0.24 min ($P < 0.01$) for helenin acetate (**4**), as compared with 0.57 ± 0.16 min in the control group; i.e., in these substances the antioxidant effect is expressed as an almost threefold increase in the latent period. It is true that this is considerably less than for Ionol at a concentration of 0.47 mM, for which $\tau = 5.05 \pm 0.78$ min. The antioxidant effect of Ionol is comparable with the effects of estafiatin (**3**) and of helenin acetate (**4**) when the concentration of Ionol is lowered to 0.19 mM ($\tau = 2.33 \pm 0.15$ min).

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Antioxidant activity was also shown by inuchinenolide C (**6**) and dideacetylinuchinenolide C (**7**), with $\tau = 1.48 \pm 0.13$ ($P < 0.001$) and 1.50 ± 0.23 min ($P < 0.01$) for the former, and 1.35 ± 0.19 min ($P < 0.01$) and 1.51 ± 0.50 min ($P < 0.2$) for the latter, respectively.

The structures of these compounds differ only by the acetyl groups, and therefore it will be interesting later to compare the AOAs of grosshemin and its acetate. Yet another lactone – grossmizin (**5**) – exhibited AOA: $\tau = 0.93 \pm 0.13$ min ($P < 0.05$).

To the compounds of the second group we assigned britanin (**8**), grosshemin acetate (**4**), α-santonin (**1**), and 3β-hydroxy-2α-senecioyloxyisoalantolactone (**2**), although AOAs have been reported in the literature for alantolactone and isoalantolactone *in vivo* [5].

Thus, five sesquiterpene lactones have proved to be good antioxidants *in vivo*, decreasing the level of the peroxide oxidation of the lipids of rat brain tissues, as was shown by an increase in the latent period of initiated CL in the presence of iron ions. Analysis of the results obtained shows the necessity for studying the dependence of AOA on the concentrations of substances and comparing it with known antioxidants *in vivo*.

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