

AMINATION OF BIOLOGICALLY ACTIVE GUAIANOLIDES CONTAINING AN  
 $\alpha$ -METHYLENE- $\gamma$ -LACTONE GROUPING

G. P. Moiseeva, I. D. Sham'yanov,  
M. R. Yagudaev, and V. M. Malikov

UDC 547.314+582.998

To elucidate the relationship between the rate of the addition reaction to the exomethylene bond of the lactone ring and the biological activity of sesquiterpene lactones a kinetic study has been made of the amination of guaianolides containing an  $\alpha,\beta$ -unsaturated lactone ring. An interrelationship has been established between biological activity, rate of the amination reaction, and the presence in the molecule of such substituents as chlorine and epoxy and ester groups.

Natural sesquiterpene lactones are selective inhibitors with high antiprotozoal and cytotoxic activity, which is connected with the presence in the molecule of such substituents as halogens and carbonyl, ester, and epoxy groups. But a particularly important condition of activity is the presence of an  $\alpha$ -methylene- $\gamma$ -lactone group. The inhibiting activity falls in the course of the addition to the exomethylene bond of the lactone ring of nucleophilic reagents containing NH and SH groups [1-4], which shows the interrelationship between the reactivity of the exomethylene bond and the biological activity of the lactone.

To elucidate the relationship between the rate of a reaction and the exomethylene bond of the lactone ring and biological activity, the kinetics of the Michael addition of diethylamine as a model nucleophile to guaianolides containing an  $\alpha,\beta$ -unsaturated lactone ring has been studied with the aid of the method of circular dichroism (CD). The guaianolides were deacetylcynaropicrin (I), chlorohyssopifolin B (II), chlorohyssopifolin A (III), acrotilin (IV), repin (V), and britanin (VI).

In the CD spectra of the guaianolides considered there is a Cotton effect in the 250-270 nm region due to a  $n \rightarrow \pi^*$  transition in the conjugated lactone chromophore. The CE is negative for the  $C_6$ -trans-linked lactones (I-V) and positive for the  $C_8$ -trans-lactone (VI) [5]. On amination, this CE disappears.

With equimolar solutions, the amination reaction took place very slowly, and, in order to accelerate it and to suppress the back-reaction in which the amination products decompose, the diethylamine was taken in excess.

The addition of an amine to a lactone is a bimolecular reaction but, since, on the one hand one of the reagents (amine) is optically inactive and its dichroic contribution to the CD spectrum was nil and, on the other hand, it was taken in large excess with respect to the lactone, the rate of the reaction was judged from the decrease in the amplitude of the lactonic CE, reflecting the change in the concentration of the initial lactone during amination, and the rate constant was calculated as for a pseudomonomolecular reaction [6]. This assumption permitted the estimation and comparison of the relative rates of amination in the series of lactones studied to be simplified. The calculation was carried out by means of the formula

$$K = \frac{1}{t} \ln \frac{H_0}{H_x},$$

where K is the rate constant;  $H_0$  and  $H_x$  are the amplitudes of the lactonic Cotton effect before and after the addition of the amine at time t, respectively; and t is the time, s.

---

Institute of the Chemistry of Plant Substances, Uzbek SSR Academy of Sciences, Tashkent.  
Translated from *Khimiya Prirodnikh Soedinenii*, No. 6, pp. 785-788, November-December, 1989.  
Original article submitted January 4, 1989.

TABLE 1

Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	$\lambda_{\max}$ , nm	Q	K · 10 <sup>4</sup> , s <sup>-1</sup>	A <sub>1</sub> , mg/ml	A <sub>2</sub>
I. Deacylcynanopicrin		=CH <sub>2</sub>	H	273	-279	8,5	5,00	—
II. Chlorohyssopifolin B	OH	CH <sub>2</sub> Cl	H	270	-373	7,5	0,50	—
III. Chlorohyssopifolin A	OH	CH <sub>2</sub> Cl		258	-2160	1,3	0,35	3,9
IV. Acroptilin				258	-1790	2,2	0,25	1,9
V. Repin				258	-1452	2,9	—	0,9
VI. Britannin				268	+370	2,5	—	15,9

\*A<sub>1</sub> is the cytotoxic activity [1], and A<sub>2</sub> is the antiprotozoal activity [2].

It can be seen from Table 1 that the nature of the substituent affects the parameters of the lactonic CE. Thus, for example, the ester group at C<sub>8</sub> in each of compounds (III-V) causes a hypsochromic shift and an increase in the intensity of the lactonic CE as compared with compounds (I) and (II) in which there is an OH group at C<sub>8</sub>. Chlorine-containing groups cause more profound changes in intensity than epoxy groups [compare compound (III) with (IV) and (V)]. The nature of the substituent at C<sub>8</sub> also exerts a great influence on the rate of addition to the exomethylene group of the lactone ring. The voluminous ester group spatially obstructs the approach of the reagent to the exomethylene bond in compounds (III-VI), and the rate of amination falls sharply in comparison with compounds (I) and (II) in which there is OH group at C<sub>8</sub>.

The rate of the reaction is also affected by the nature of the substituents in ring A, as can be seen from a comparison of K for compounds (III) and (IV) and (I) and (II).

A comparison of the rate constants and cytotoxic activities of chlorohyssopifolin B and chlorohyssopifolin A, which differ only by the substituent at C<sub>8</sub>, showed that although addition to the lactonic exomethylene took place faster in the case of chlorohyssopifolin B, chlorohyssopifolin A possessed a more powerful inhibiting action; this can be explained by the presence of the chlorine-containing group at C<sub>8</sub>, which enhances the lipophilic properties of chlorohyssopifolin A. A comparison of compounds (I) and (II), differing only by the substituents in ring A, also showed that compound (II), thanks to the presence of a chloromethyl group at C<sub>4</sub>, possessed a higher cytotoxic activity in spite of the fact that its rate of amination was greater than that of (I). The replacement of the chloromethyl group by an epoxy group raised the rate constant and biological activity, as can be seen from a comparison of compounds (III) and (IV) and of (IV) and (V).

Thus, an analysis of the results obtained showed that the presence of such structural elements as epoxy, chloromethyl, and ester groups is a more important factor in raising biological activity than the rate of addition to the exomethylene bond of the lactone ring.

A parallelism between the rate of amination of the exomethylene group of the lactone ring and biological activity was traced only in that case when the nature of the linkage of the lactone ring was the same and the stereochemical environments were relatively monotypical. Among the compounds studied, chlorohyssopifolin A, acroptilin, and repin correspond to these

demands, and in these, as can be seen from Table 1, with an increase in the rate constant of addition to the exomethylene group of the lactone ring a rise in biological activity was also observed.

#### EXPERIMENTAL

CD spectra were recorded on a JASCO J-20 spectropolarimeter in a thermostated cell 1 cm long at a temperature of 25°C. Ethyl alcohol was used as solvent. Because of the low solubility of the guaianolides studied, dilute - 0.003 M - solutions of the lactones were used. Before mixing, the solutions of the lactones and the diethylamine were kept in a thermostat at 25°C.

To 1 ml of an alcoholic solution of a lactone was added 0.03 ml (19.5 mg) of diethylamine, and the mixture was carefully stirred and was rapidly transferred into the thermostated cell. After the addition of the amine, the intensity of the lactonic CE was measured every 15 min for 1.5 h at the wavelength corresponding to the maximum of the lactonic Cotton effect.

Information on biological activity was taken from the literature [1, 2].

#### SUMMARY

1. It has been established that the nature and position of substituents affect the parameters of the lactonic Cotton effect and the rate of the amination of lactones.

2. The presence of such structural elements as epoxy, chloromethyl, and ester groups is a more important factor for raising biological activity than an increase in the rate of addition at the exomethylene group of the lactone ring.

3. A direct relationship has been observed between the rate of amination and biological activity only in the case where the linkages of the lactone rings are the same and their stereochemical environments are relatively monotypical.

#### LITERATURE CITED

1. A. G. Gonzales, V. Darias, G. Alonso, and E. Estevez, *Planta Med.*, **40**, 179 (1980).
2. A. M. Rubinchik, K. S. Rybalko, R. I. Evstratova, and O. A. Konovalova, *Rast. Res.*, **12**, No. 2, 170 (1976).
3. S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe, *Science*, **168**, 376 (1970).
4. S. M. Kupchan, T. J. Giacobbe, J. S. Krull, A. M. Thomas, M. A. Eakin, and D. C. Fessler, *J. Org. Chem.*, **35**, No. 10, 3539 (1970).
5. T. G. Waddell, W. Stocklin, and T. A. Geisman, *Tetrahedron Lett.*, 1313 (1969).
6. C. D. Nenitescu, *General Chemistry* [Russian translation from the Romanian], Mir (1968), Moscow, p. 272.