

Chemistry and biotransformations in the Scrophulariaceae family*

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Abstract: One of our research lines has been the chemical studies among *Calceolaria* species (Scrophulariaceae), from which have been isolated 77 new diterpenes of various skeleton types (labdanes, pimaranes, stemaranes, and stemodanes), all coming from a common bicyclic intermediate, having H-9 and C-20 as a *cis* relationship and C-11 becomes *trans* with respect to C-20. Another line of research is bringing out chemical changes on diterpenes obtained from this family, using biological systems as the fungus *Gibberella fujikuroi* and *Mucor plumbeus*.

About 200 genera belong to the Scrophulariaceae family, and about 4900 species are distributed all over the world, especially in medium-temperature regions [1]. Only the third part of these genera have been chemically studied, and iridoids, phenylpropanoids, saponines, cardiotonic glycosides, flavanoids, alkaloids, and terpenes have been isolated. Many of these compounds have interesting biological activities [2].

Six genera are represented in Chile, and among these *Calceolaria* is the most abundant with 86 species and *Stemodia*, which has only one species [3].

During 15 years, our research has allowed us to isolate 77 new diterpenes from different species of the genus *Calceolaria* and 5 diterpenes from the species *Stemodia chilense*. These diterpenes are of various types of skeletons, i.e., labdane, abietane, pimarane, stemarane, stemodane, and thyriflorane.

In the chemical study of *Calceolaria glandulosa* [4] we have found diterpenes of the pimarane skeleton, 18 hydroxy-9-*epi-ent*-isopimara-7,15-diene **1**, in which a methine carbon appears at an unusually upfield position (δ 38 ppm); this fact made that a normal isopimaradiene structure was discarded, because apart from the known endocyclic homoallylic effect of the Δ^7 bond [5] and the γ -gauche effect due to the C-18 oxymethylene group, another interaction must operate in order to justify this unusually upfield absorption of C-5 (normal value at δ 50 ppm). This effect could be explained by the interaction imposed by an uncommon orientation of C-11, which also produced an additional γ -effect on C-5; it also produces a shielding effect on C-1, as well as a deshielding on C-20. In order to remove any ambiguity concerning the structure and stereochemistry of compound **1**, it was subjected to X-ray analysis.

9-Epimeric diterpenoids constitute a relative rare group of secondary metabolites with only a few examples known prior to our work, i.e.,annonalide [6], momilactones A-C [5,7].

From a biogenetic point of view, the formation of all the diterpenes found in *Calceolaria* species involves an enzymatic activity conducting toward a “chair–boat” cyclization of geranyl–geranyl

*Lecture presented at the 22nd IUPAC International Symposium on the Chemistry of Natural Products, São Carlos, Brazil, 3–8 September 2000. Other presentations are published in this issue, pp. 549–626.

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pyrophosphate, as opposed to the normal “chair–chair” cyclization in the construction of the bicyclic intermediate [8].

When the atypical “chair–boat” cyclization occurs, H-9 and C-20 adopt a *cis* relation and C-11 becomes *trans* with respect to C-20. This fact can also explain the formation of the other types of skeletons, i.e., labdanes [9], stemaranes [10], thyrifloranes [11], and stemodanes [12].

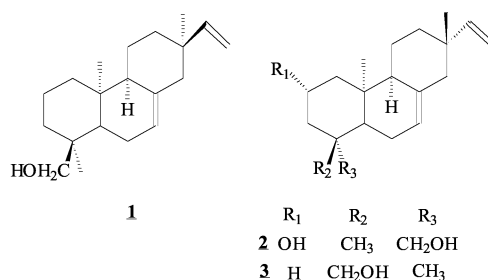
In *C. sessilis* [13] we have isolated another type of compound, three naphthoquinones, which were tested against *Trypanosoma cruzi* epimastigotes and tumor cells, and the most active compound was (-)-2,3,3-trimethyl-2,3-dihydronaphtho[2,3-b]furan-4,9-quinone; the 50% culture growth inhibition (I_{50}) on *T. cruzi* was at concentrations ranging from 2.1 to 5.2 μ molar, it also inhibited TA3 and TA3-MTX-R culture growth with an I_{50} of 2.1 and 3.8 μ molar, respectively. Another activity encountered for this naphthoquinone was that it inhibited the respiration of the tumor cells by interfering with the electron transport at some point between NADH and ubiquinone, but did not inhibit the respiration of *T. cruzi*, and produced a temporary increase of oxygen consumption in *T. cruzi* and tumor cells, suggesting the generation and participation of free radicals [14].

Since 1997, we have developed a new research line, that is, the use of biological systems to bring about chemical changes on compounds that are not their natural substrates. These biotransformations have a number of advantages when viewed alongside the corresponding chemical methods: they are not only regio- and stereospecific but are also enantiospecific, allowing the production of chiral products from racemic mixtures, and it is possible to obtain biotransformations at centers that are chemically unreactive, and there is a topological relationship between the substrate and the active site of the enzyme. Microbial processes have been used to introduce hydroxyl groups at difficult positions on diterpenoid compounds [15]. Chemical–microbiological methods constitute an alternative way to obtain new polyoxygenated compounds from abundant products isolated.

The giberellins are a group of widely distributed plant hormones that participate in the regulation of the growth and development of higher plants [16]. These compounds have also been isolated from fungi such as *Giberella fujikuroi*. While they possess a common carbon skeleton, they differ mainly from one another in their hydroxylation pattern. Since in the formation of *ent*-kaurene, precursor of the giberellins, an *ent*-pimarane carbonium ion has been proposed as an intermediate [17], we believe that biotransformations of compounds with this carbon framework to be of particular interest, so we started the study of biotransformation of *ent*-pimaranes isolated from *Calceolaria* species.

The first diterpene submitted to biotransformation with the fungus *Giberella fujikuroi*, was 2 α ,19-dihydroxy-9-*epi-ent*-pimara-7,15-diene **2**, isolated from *C. hypericina* [18], and the results showed that the main compound obtained in the biotransformation, was formed by epoxidation of the 7,8 double bond of the substrate. This was followed by allylic hydroxylation at either C-6(β) or C-9(β). The alternative route, whereby hydroxylation precedes epoxydation, cannot be disregarded [19].

The incubation of 18-hydroxy-9-*epi-ent*-pimara-7,15-diene **3**, isolated from *C. petioalaris* [20] with *G. fujikuroi*, gave as the main reaction observed, the epoxidation of the 7,8 double bond of the substrate and the allylic hydroxylation at either C-6(β) or C-9(β). The reactions observed in this feeding are similar to those produced in the incubation of 2 α ,19-dihydroxy-9-*epi-ent*-pimara-7,15-diene, which



implies that a replacement of a 2 α - and a 19-hydroxy by a 18-hydroxy has had few effects on the results of these biotransformations. This fact indicates a lack of specificity of the enzymes involved in these processes [21].

The results of the incubation of 18 hydroxy-9-*epi-ent*-isopimara-7,15-diene **1**, isolated from *C. glandulosa* [4], showed the formation of compounds epoxidated at the 7,8 double bond of the substrate, which rapidly suffer transpositions to form the oxoderivatives and compounds with hydroxylation at C-11.

(+)-2-Deoxy-stemodinone isolated from *Stemodia chilensis* [22], was incubated on the fungus *Mucor plumbeus*, and the main compounds are oxydated on ring A.

It may be pointed out that the importance of these studies is, in the first place, to establish the chemical constitution of the Scrophulariaceae family, to test if some metabolite has an active principle and, finally, to develop synthetic products by means of biotransformations with the aim to obtain molecules with higher oxydation, approaching in this manner to molecules of known activity

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