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Antiproliferative Activity and Effect on GABAA Receptors of the Abietane Diterpenoid Jiadifenoic Acid C and Other Callitrisic Acid (4-Epidehydroabietic Acid) Derivatives

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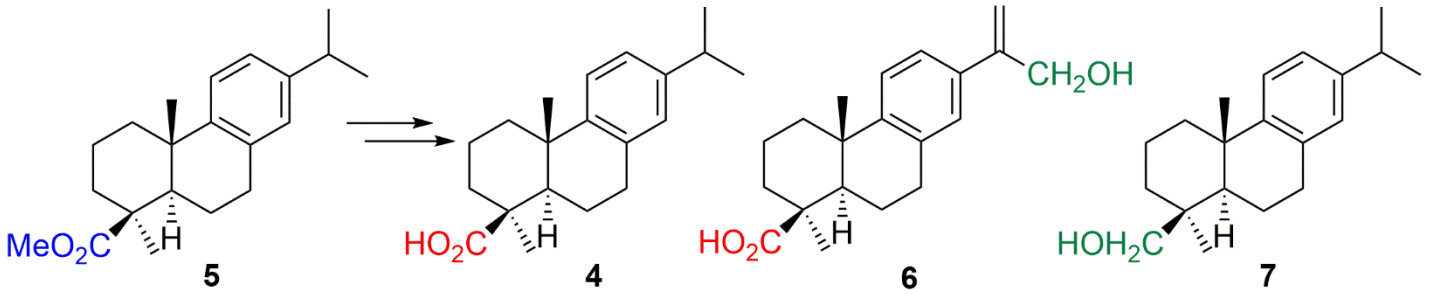


	5	**4**	**6**	**7**
Potentiation I_{GABA} (%) at 100 μM	-	269	-	311
GI_{50} (μM) for A549, HBL-100, HeLa, SW1573, T-47D,WiDr	6.4-17	16-36	19-61	3.4-18

Abstract: The **abietane**-type and related diterpenoids are a class of naturally occurring terpenoids in the plant kingdom, which have demonstrated a wide range of biological activities against cancer, and a variety of infectious diseases (viral and bacterial). For instance, **dehydroabietic acid** (DHA) has been studied for its pharmacological properties, including several synthetic programs of derivatization. However, its C4-epimer, 4-epidehydroabietic acid or **callitrisic acid**, has been little studied biologically so far. Recently, we reported a study for the multi-gram isolation of the methyl ester of this acid from Sandarac resin which led to the semisynthesis of bioactive **jiadifenoic acid C**. This research has allowed further studies on this molecule as well as related derivatives.

In this study, we have synthesized jiadifenoic acid C as well as callitrisic acid and callitrisinol from methyl callitrisate for further biological studies. In particular, the **antiproliferative** activity (GI50= 3.4-61 μM) against a panel of six representative human solid tumor cell lines and the effect on **GABAA receptors** (potentiation of I_{GABA} 269-311% at 100 μM) was evaluated. We were interested in changes in the bioactivity of the C19-functionalized callitrisic acid derivatives in comparison with the activity in C18-functionalized dehydroabietic acid and similar derivatives.

Keywords: Abietane Diterpenes; jiadifenoic acid; callitrisic acid; antiproliferative; GABAA receptor modulators.

Introduction

At present, about fifty percent of commercial pharmaceutical drugs are derived from natural sources. The abietane-type and related diterpenoids are a class of naturally occurring terpenoids in the plant kingdom,[1] which have demonstrated a wide range of biological activities against cancer, and a variety of infectious diseases (viral and bacterial).[2]

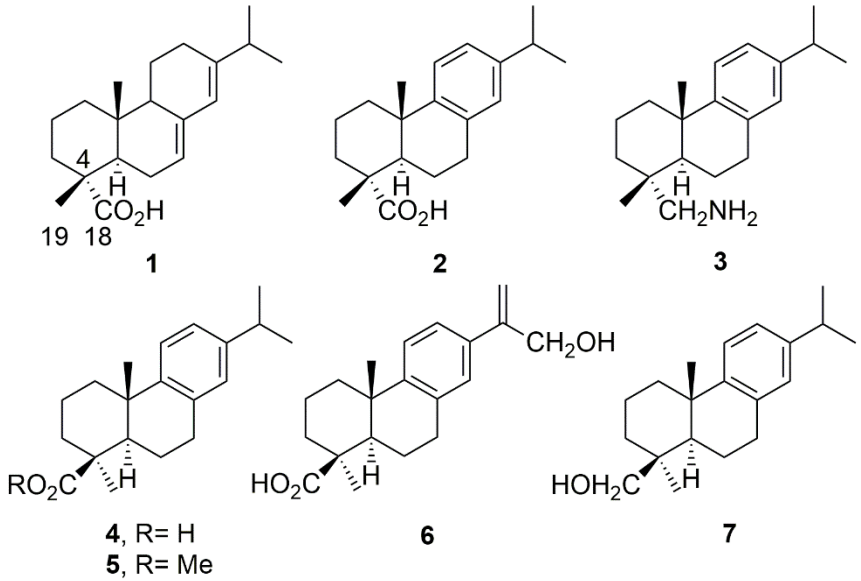


Figure 1. Common starting materials and tested compounds.

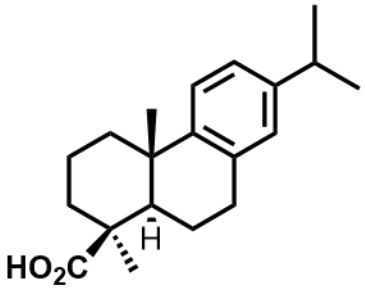
Several research groups have explored the potential as chemotherapeutic agents of abietanes by means of semisynthetic derivatives from **abietic acid** (**1**)-derived materials such as **dehydroabietic acid** (**2**, DHA), and **dehydroabietylamine** (**3**), also called leelamine (**Fig. 1**).[2] For example, DHA displays not only antiulcer and antimicrobial properties, but also antitumor effects. Recently, DHA was reported as a positive GABAA receptor modulator inducing significant receptor modulation in the oocyte assay, with a maximal potentiation of I_{GABA} of 682.3% ± 44.7% at 100 µM.[3]

Introduction

DHA displays an equatorial carboxylic group located at C18 while in other natural congeners the carboxylic group adopts the axial configuration (C19) as in 4-epidehydroabietic acid or callitrisic acid (**4**) (**Fig. 1**). Callitrisic acid (**4**) is a diterpenoid acid contained in the resins of several *Callitris* species (Cupressaceae). This acid also occurs in plants of the genus *Juniperus*, *Calceolaria* and *Illicium*. Its biological properties have not been studied in deep, especially those of their derivatives due to limited availability of the parent acid.

Callitris sp.

Sandarac Resin



Callitrisic acid

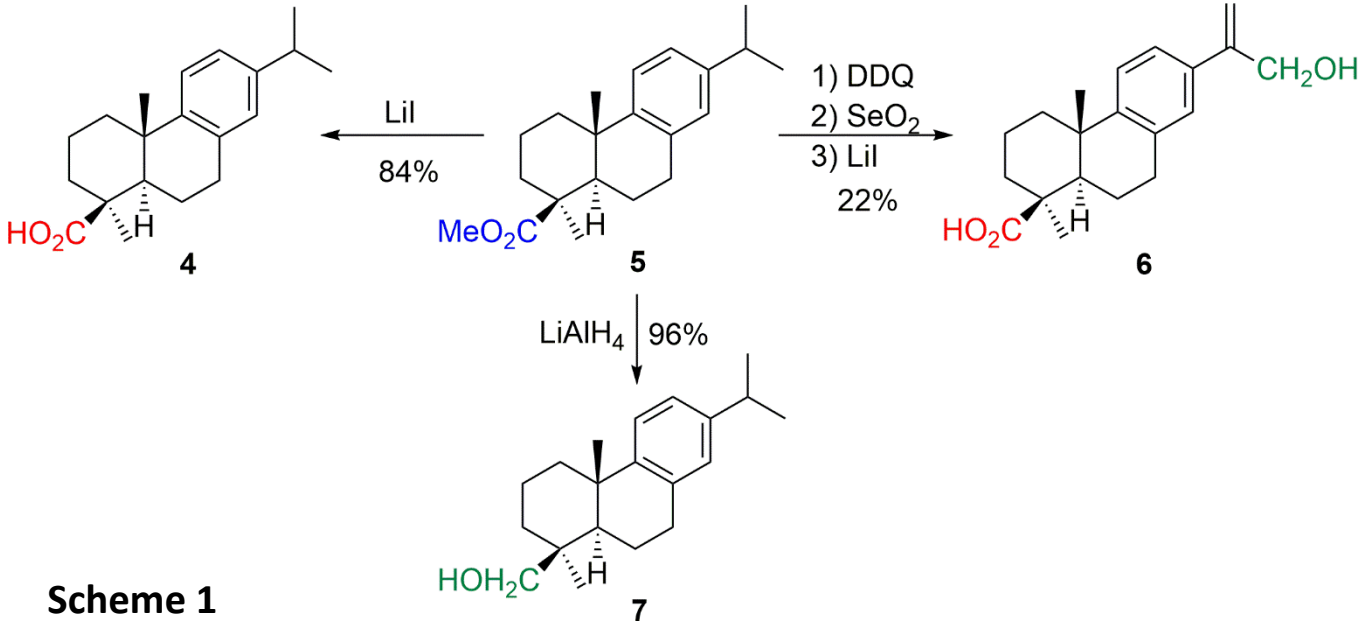
González, M. A.; Zaragozá, R. J. *J. Nat. Prod.* **2014**, *77*, 2114-2117

Introduction

Recently, a series of related acids having a C19 carboxylic group have been isolated such as jiadifenoic acids A-I. These, including callitrisic acid, have shown important antiviral properties against Coxsackie virus. We have recently developed the synthesis of jiadifenoic acid C (**6**, **Fig. 1**) from methyl callitrisate (**5**) isolated from Sandarac resin. The ready availability of these materials from our studies (**5-6**) and the absence of their biological studies as well as chemical manipulation prompted us to carry out this research. Herein, we report the evaluation of callitrisic acid (**4**) and derivatives **5-7** (**Scheme 1**) against a panel of six representative human solid tumor cell lines and their effect on GABAA receptors (α1β2γ2s), with the aim of studying the influence of stereochemistry at C4 in the biological activities.

Results and discussion

The compounds were obtained from methyl callitrisate (**5**), which was obtained from commercially available Sandarac resin, as outlined in Scheme 1. The synthesis of jiadifenoic acid C (**6**) starts with the regioselective dehydrogenation of methyl callitrisate (**5**) by treatment with 2,3-dichloro-5,6-dicyanoquinone (DDQ), followed by allylic oxidation with catalytic selenium dioxide and tert-butyl hydroperoxide (TBHP) as co-oxidant and finally, nucleophilic methyl ester cleavage with LiI to afford jiadifenoic acid C (**6**) in 22% overall yield (**Scheme 1**). The same ester cleavage on methyl callitrisate (**5**) afforded callitrisic acid (**4**) in 84% yield while reduction with LiAlH4 gave callitrisinol (**7**) in 96% yield (**Scheme 1**).



Scheme 1

Results and discussion

With compounds **4-7** in hand, the antiproliferative activity against six representative human solid tumor cell lines: A549 (lung), HBL-100 (breast), HeLa (cervix), SW1573 (lung), T-47D (breast), and WiDr (colon) was studied using the SRB assay. The results expressed as GI50 are given in **Table 1**.

Table 1. Antiproliferative activity (GI50, μM) of callitrisic acid derivatives **4-7** against human solid tumor cells.

	Cell line (origin)					
compound	A549 (lung)	HBL-100 (breast)	HeLa (cervix)	SW1573 (lung)	T-47D (breast)	WiDr (colon)
4	16	36	15	32	33	31
5	10	14	16	17	8.8	6.4
6	19	61	27	55	37	47
7	11	17	13	18	10	3.4
etoposide	-	1,4	3,3	15	22	23
cisplatin	4,9	1.9	1,8	2,7	17	23

Results and discussion

All compounds were active (GI50 < 100 μM,) in the cell lines tested, being jiadifenoic acid C (**6**) the least potent compound with GI50 values in the range 19-61 μM against all cell lines, while compounds **5** and **7** were the most potent at a similar level in all cell lines. In particular, compound **7** was the most potent against WiDr cells (GI50 = 3.4 μM) while compound **5** was the most potent against T-47D cells (GI50 = 8.8 μM), being 6.7- and 2.5-fold more potent than the reference compound etoposide, respectively. In general, the order of activity in the callitrisic series with different functional groups at C19 was alcohol ≥ ester > acid. Our previous study on the biological activity of dehydroabietic acid derivatives (cytotoxicity in HeLa and Jurkat cell lines by MTT) was also consistent with this order of activity.[4] Though the GI50 values obtained with the SRB assay and IC50 values obtained with the MTT assay are not fully comparable, it is worth to note that the dehydroabietic acid derivatives (C18-functionalized) in our previous study gave IC50 values in the range 13-101 μg/mL (IC50 = 45-337 μM) for HeLa cells. Thus, it can be concluded that our C19-functionalized callitrisic series (**4**, **5**, and **7** GI50 = 15, 16, and 13 μM, respectively) for HeLa cells was more potent than the corresponding C18-functionalized series. Also, a SAR trend is that a C19 hydroxymethyl group produced the best antiproliferative activity, whereas a C19 carboxylic group led to less active compounds including jiadifenoic acid C (**6**) as the least potent. Thus, the presence of an allylic alcohol at C13 seems to be detrimental for antiproliferative activity.

Results and discussion

In another set of experiments, compounds **4-7** were tested for their effects on GABAA receptors (α1β2γ2s) by means of the two-microelectrode voltage clamp technique in *Xenopus laevis* oocytes. The results are summarized in **Table 2**.

Table 2. Potentiation of IGABA in α1β2γ2s receptors by compounds **2** and **4-7**.

Compound	Potentiation of I_{GABA} (%) at 10 μM	Potentiation of I_{GABA} (%) at 100 μM
2	192	789
4	14	269
5	N.A[a]	N.A[a]
6	N.A[a]	N.A[a]
7	116	311

[a] N.A.: not active

Results and discussion

Callitrisinol (**7**) modulated IGABA at 10 and 100 μM (potentiation of IGABA of 116% and 311%, respectively), while methyl callitrisate (**5**) and jiadifenoic acid C (**6**) were inactive. Callitrisic acid (4) only enhanced GABA evoked currents at 100 μM (potentiation of IGABA of 269%). Thus, it can be concluded that the presence of an allylic alcohol at C13 reduced significantly the GABAA receptor modulating effect, while the presence of a C19 hydroxymethyl group increases activity. On comparing GABAA modulating activity of DHA (**2**) (potentiation of IGABA of 789% at 100 μM) with its C4-epimer, callitrisic acid (**4**) (potentiation of IGABA of 269% at 100 μM), it can be concluded that the stereochemistry at C4 is very important for activity, being the callitrisic acid series the least active.

Conclusions

In summary, the compounds in this communication support importance of the aromatic abietanes with dehydroabietane skeleton for antiproliferative activity. Despite the small set of compounds, the role of the stereogenic center at C4 for antiproliferative and GABAA receptor modulating activities has been revealed. Further studies to identify more structure-activity relationships and enhance the observed activities are under way.

Acknowledgments

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