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New acronycine derivatives with increased antitumour activity

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ABSTRACT Acronycine cis-diol diacetate is an example of acronycine drug, a potent antitumour agent. This drug is now under preclinical development.

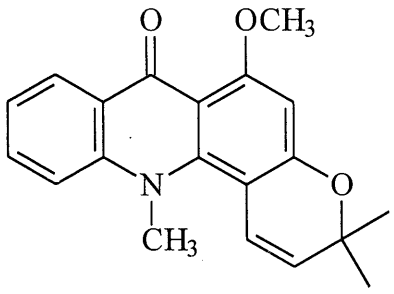
ABSTRAK Akronisina cis-diol diasetat adalah satu contoh drug akronisina, anti-barah yang poten. Sekarang drug ini dalam kajian preklinikal.

(Acronycine, Antitumor, Rutaceae, *Acronychia baueri*, NMR)

INTRODUCTION

Acronycine is a natural alkaloid first isolated in 1948 by the group of Hughes and Lahey [1] from the bark of an Australian Rutaceae, *Acronychia baueri*. Since that time, the position of this plant within the Rutaceous family has been revised several times by Hartley [2, 3], at the Herbarium Australiense. Now he considers the plant belongs to the genus *Sarcomelicope* and has revised its name to *Sarcomelicope simplicifolia*.

The structure of acronycine **1** has been a subject of lenghty discussion, mainly to know if the pyran ring was fused linearly or angularly on the acridone skeleton. It is only in 1966 that the angular structure could be unambiguously assigned to acronycine on the basis of NMR data [4].



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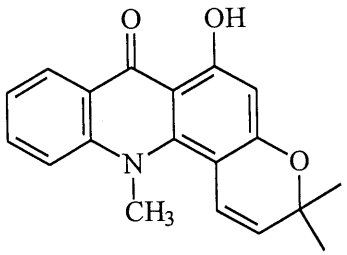
The biological interest of acronycine was discovered in 1966 by Gordon Svoboda in the Eli Lilly Laboratories [5,6]. He demonstrated that acronycine was a potent anticancer agent. Its main interest lies in its broad spectrum of activity which include numerous solid tumours resistant to other chemotherapeutic agents such as various sarcoma, carcinoma and melanoma. However, acronycine exhibits only a marginal activity against leukemias.

Up to now, the clinical trials of this compound have been severely hampered, both due to its moderate potency and also to very low water solubility (less than 1 mg per liter). Most of the trials have therefore relied upon oral administration and have had to be interrupted due to very poor gastrointestinal tolerance, causing vomiting and diarrhea [7].

More recently, the group of Dorr in the College of Pharmacy of the University of Arizona [8] succeded in solubilizing acronycine in the cosolvent used for etoposide in human therapeutics. The trials were realized intraveinously on nude mice xenografted with human solid tumours, such as breast cancer or multiple myeloma. In all cases, acronycine exhibited a very interesting activity which could be compared with that of melphalan used as positive control during these tests. These experiments demonstrate the great potential interest of acronycine.

Since the discovery of the antitumor properties of acronycine, about one hundred derivatives and structural analogues have been prepared and tested either for their cytotoxic activity *in vitro* or for their antitumor properties *in vivo*. As far as structure activity relationships are concerned, it is clear that the chain which etherifies the oxygen at C-6 can be modified without altering the activity. Several derivatives possessing a basic chain in that position have been prepared with the aim of obtaining water soluble salts [9]. Unfortunately, such salts are very unstable and readily decompose, in acidic medium, into noracronycine **2** a biologically inactive compound bearing a free chelated phenolic group. In a similar way, 1,2-dihydroacronycine **3** is also devoid of antitumor activity [5].



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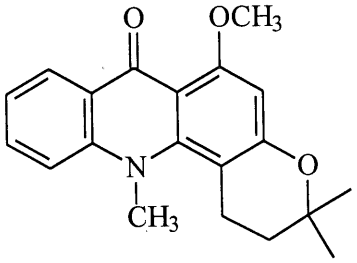

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RESULTS AND DISCUSSION

This report will discuss a series of acronycine analogues recently synthesized in our laboratory which revealed some interesting discoveries. One of the very first problems we wanted to explore in that series was the exact role of the substituent at C-6. In fact, the lack of activity of noracronycine **2** could be either due to a reduction of the steric hindrance in that position or to the dramatic change in the electron repartition within the aromatic structure due to chelation of OH-6 by the neighbouring carboxyl group. In that sense, 6-demethoxyacronycine was a very good prospect, with a highly reduced steric hindrance at C-6 but an electronic repartition not too different from that of acronycine itself. We succeeded in synthetizing this compound in seven steps and 20 % overall yield according to Scheme 1, which the key steps are (i) alkylation of 3-nitrophenol by a chloropropargylic derivative, (ii) selective iron reduction of the nitro function, (iii) Claisen rearrangement with an excellent regioselectivity, (iv) obtention of a carboxylic diphenylamine through Ulmann reaction, (v) ring closure using trifluoroacetic anhydride, followed by methylation [10]. 6-Demethoxyacronycine was tested against the proliferation of various tumor cells *in vitro*, namely against fibroblastic chinese hamster lung cell lines DC-3F (both wild and actinomycine resistant) and against the classical murine leukemia L 1210. In both cases, 6-demethoxyacronycine exhibited an activity which is comparable with that of acronycine itself (Table I), suggesting the electron repartition phenomena was more important than the steric hindrance at C-6 for the biological activity.

Table I. Compared activities of 6-demethoxyacronycine and acronycine

	Acronycine	**6-Demethoxyacronycine**
DC-3F	$1C_{50}$=11.25μM	$1C_{50}$=8.75μM
DC-3F/ADX	$1C_{50}$=3.5μM	$1C_{50}$=2.75μM
L1210	$1C_{50}$=27μM	$1C_{50}$=29.9μM

Another possibility which was explored simultaneously in our group was the search for new natural pyranoacridones in Rutaceous plants which could help us in obtaining new active acronycine derivatives. Based on the chemotaxonomic studies, such alkaloids are likely to be present in species belonging to the genus *Sarcomelicope*, related to the Australian plant from which acronycine had been first isolated. If one analyse the distribution map of *Sarcomelicope* species, one can observe that all the species of that genus are endemic to New Caledonia, except *Sarcomelicope simplicifolia* which is widely distributed, from Australia to Fidji Islands [3, 4]. Thus, through the French (CNRS) mission in New Caledonia, the study of alkaloids from the *Sarcomelicope* species was made possible.

Table II shows the species and subspecies of *Sarcomelicope* which have been botanically described until present. All the species highlighted by an asterisk have been studied in our laboratory for their alkaloidal contents. In addition, one of the remaining ones, *Sarcomelicope follicularis* is now currently under study. Most of the species led us to the isolation of novel alkaloids but only those which have the novel angular pyranoacridones related to acronycine shall be discussed.

Table II. Species and subspecies of the genus Sarcomelicope

• *Sarcomelicope simplicifolia* (Eddl.) Hartley
• *Sarcomelicope simplicifolia* (Eddl.) Hartley ssp. *neoscotica.* (*) (P.S. Green) Hartley
• *Sarcomelicope simplicifolia* (Eddl.) Hartley ssp. *petiolaris.* (A. Gray) Hartley
• *Sarcomelicope argyrophylla* Guill. (*)
• *Sarcomelicope glauca* Hartley. (*)
• Sarcomelicope *dogniensis* Hartley. (*)
• Sarcomelicope *sarcococca* (Baill.) Engler.
• Sarcomelicope *leiocarpa* (P.S. Green) Hartley. (*)
• Sarcomelicope *follicularis* Hartley.
• Sarcomelicope *pembaiensis* Hartley. (*)
• Sarcomelicope *megistophylla* Hartley. (*)

(*) Species chemically studied in our laboratory.

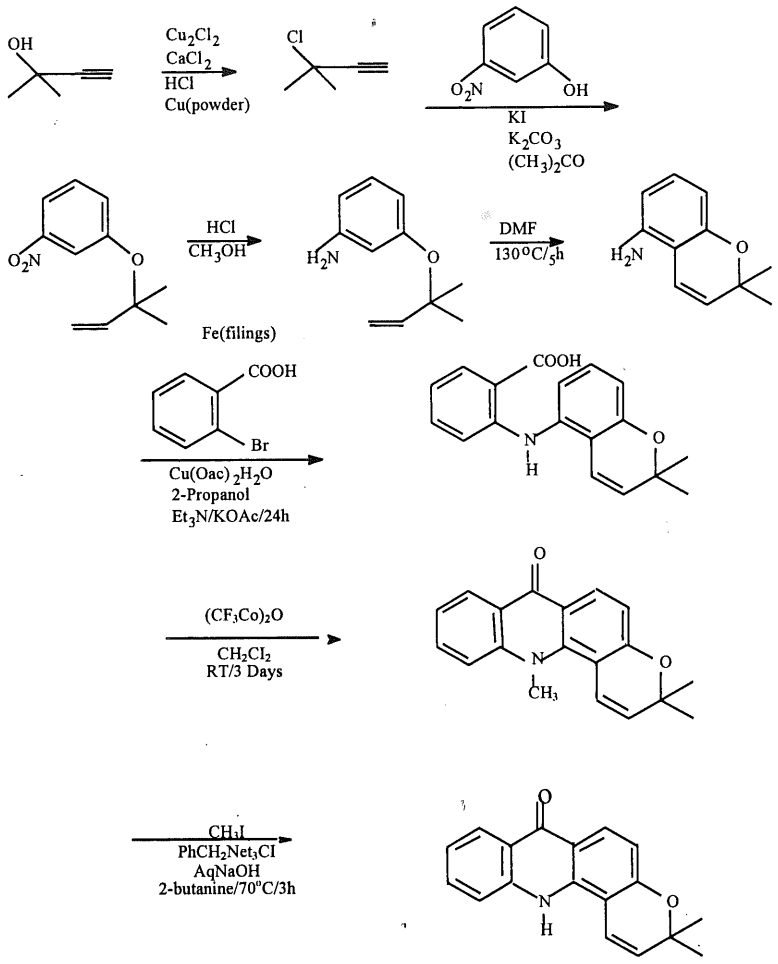
The major alkaloid isolated from the leaves of *Sarcomelicope dogniensis* is N-desmethyl-acronycine. Several alkaloids differing slightly on the pyran ring were also obtained namely:

1-hydroxy-1,2-dihydro-N-desmethyla-cronycine **4**,

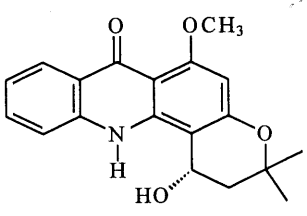
1-oxo-1,2-dihydro-N-desmethylacrony-cine **5**,

cis-1,2-dihydroxy-1,2-dihydro-N-desmethylacrony-cine **6**.

In addition, a base with a cleaved pyran ring was also isolated named 1-methoxy-3-(2-methyl-propanal-2-oxy)-acridin-9-one-4-carbaldehyde **7**.



Scheme 1. Synthesis of 6-demethoxyacronycine



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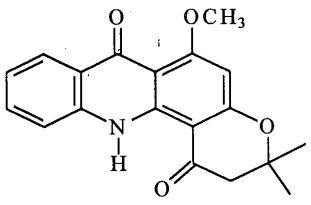

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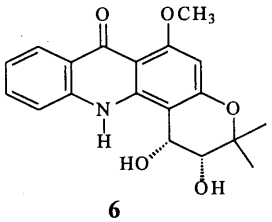

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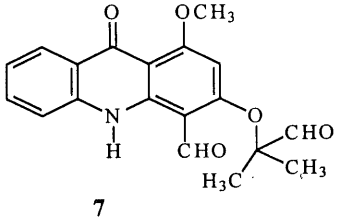

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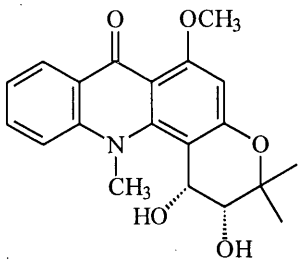
All these compounds could be readily synthesized from N-desmethylacronycine to ensure their structures. For instance, treatment of N-desmethylacronycine with osmium tetroxide led to the corresponding *cis*-diol which could be oxidized by periodate to yield the open compound, whereas 1-hydroxy-1,2-dihydro-N-desmethylacronycine and 1-oxo-1,2-dihydro-N-desmethylacronycine could be obtained through the intermediacy of a bromohydrine derived from N-desmethyl-acronycine [11].

The alkaloidal contents of *Sarcomelicope glauca* was even more interesting. Its main alkaloid was acronycine itself. Two oxidation products were also present in the plant, namely: *cis*- and *trans*-1,2-dihydroxy-1,2-dihydroacronycine **8, 9** [12].



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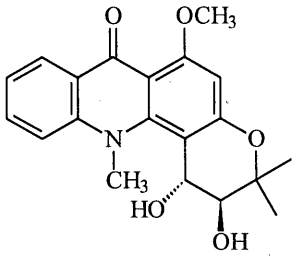

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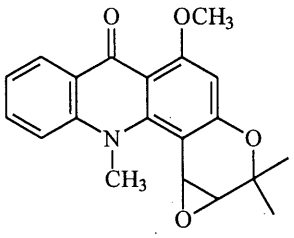
These two natural alkaloids were optically active and resulted from an enzymatic oxidation of acronycine in the plant. We postulated the first step of this oxidation was most probably the formation of an epoxide which could then be hydrated.

This compound, acronycine epoxide **10**, could finally be isolated in very minute amounts from several *Sarcomelicope* species and it was firstly obtained from *Sarcomelicope argyrophylla* [13].

It is a highly unstable compound and all our attempts towards its synthesis were unsuccessful. In fact, formation of this epoxide can be ascertained by both TLC and NMR when acronycine is treated with an oxydant such as 3-chloroperbenzoic acid, but its isolation from the reaction mixture was unsuccessful, due to its high reactivity.



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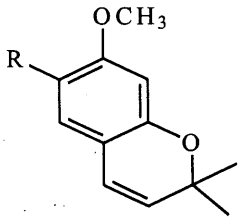
This led the author to consider this epoxide as a possible active metabolite of acronycine *in vivo*, which should react on nucleophilic targets within the tumor cells.

This hypothesis was in agreement with what was known about the only biologically active chromenes previously described, the precocenes **(11&12)**. These simple chromenes, first isolated from the Compositae species *Ageratum houstonianum* by Bowers exhibit a broad spectrum of insecticidal activities [14].

In sensitive species, precocene metabolites are the corresponding *cis* and *trans* dihydrodiols which accumulate in the *corpora allata* of the insects.



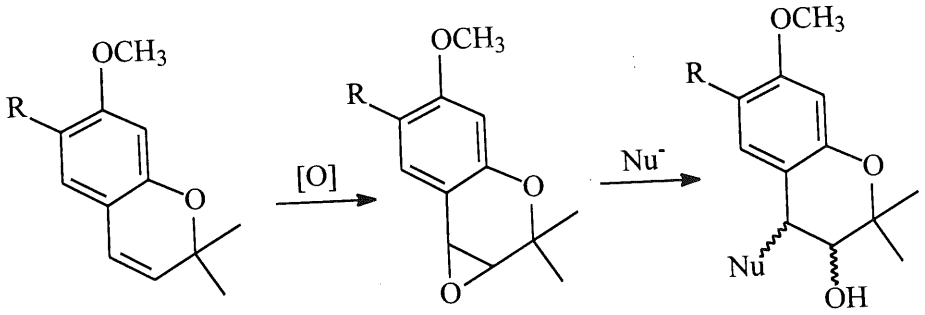
11, R= H

12, R= OCH_3

The *Corpora allata* are the organs responsible for the biosynthesis of juvenile hormone in insects and the final step of its biosynthesis involves the oxidation of a double bond to the corresponding oxirane mediated by a specific enzyme using cytochrome P450 as coenzyme. Bowers could demonstrate that in sensitive

insects, precocenes could be accepted as substrates by this enzyme, leading to the highly reactive precocene epoxides which were responsible for alkylation of nucleophiles present in biological matrices such as cellular proteins, causing membrane alteration at the sub-cellular level and ultimately irreversible damages and cell death [14-17].

These data about precocenes and the crucial role of the pyran double bond in the acronycine series, where 1,2-dihydroderivatives are biologically inactive, were in good agreement with the postulate that acronycine epoxide could be the active metabolite of acronycine *in vivo*. Nevertheless, the great unstability of acronycine epoxide and, for instance, its fast reaction with water to yield the corresponding diols excluded its possible use as an anticancer drug. With the goal of finding new antitumor candidates having a better stability than acronycine epoxide but a similar reactivity towards nucleophilic agents, we decided to synthetize a series of *cis*-1,2-dihydroxy-1,2-dihydro diesters in both acronycine and 6-demethoxyacronycine series.

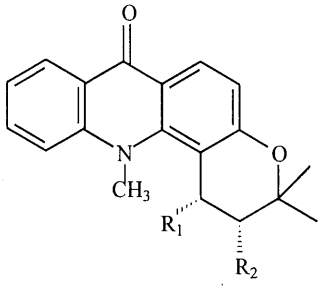


Scheme 2

In both series, the diols could be readily obtained in excellent yield by catalytic osmic oxidation of either acronycine or 6-demethoxyacronycine [18-20]. The corresponding diesters could be prepared easily from these diols (Scheme 2). An excess of acid anhydride leads to homogenous diesters exemplified by the diacetates obtained upon action of acetic anhydride. The successive use of one equivalent of a first anhydride and then of an excess of a second anhydride enables to prepare mixed esters such as the acetobenzoates. Finally, the use of N, N'-carboxyldiimidazole or similar reagents permitted us to obtain cyclic esters such as carbonates.

In the 6-demethoxyacronycine series, the biological results of the cytotoxicity tests against L1210 leukemic cells were very disappointing since all the compounds were merely inactive (Table III), suggesting the mechanism of action of acronycine and 6-demethoxyacronycine at the molecular leve should be different. In contrast, as we expected the results were very interesting in th acronycine series. All the diesters, which wer tested exhibited a strong cytotoxicity agains L1210 cells i*n vitro*, fairly higher than that c acronycine itself which is not very activ against leukemias (Table IV).

Table III: *In vitro* cytotoxicity of 1,2-dihydroxy-1,2-dihydro-6-demethoxyacronycine diesters



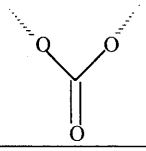
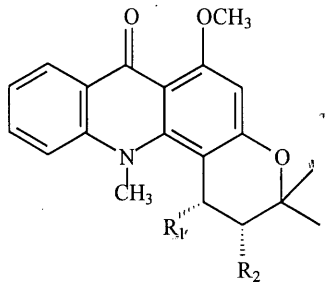
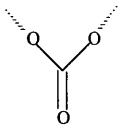
R_1	R_2	L1210 $IC_{50}\mu M$
6-Demethoxyacronycine		29.9
OH	OH	>50
$OCOCH_3$	$OCOCH_3$	>50
		>50

Table IV: *In vitro* cytotoxicity of 1,2-dihydroacronycine diesters



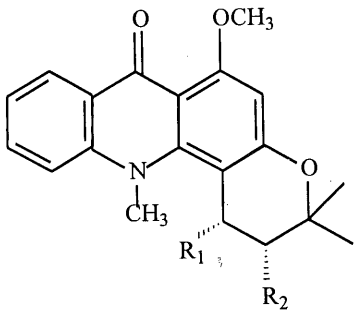
R_1	R_2	L1210 $IC_{50}\mu M$
Acronycine		27
H	H	34.4
OH	OH	80.6
OH	$OCOCH_3$	8.9
$OCOCH_3$	$OCOCH_3$	4.9
		0.24
OH	$OCOC_6H_5$	7.1
$OCOCH_3$	$OCOC_5H_5$	5.0

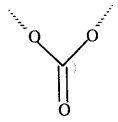
These encouraging results prompted us to test these compounds *in vivo* both against murine leukemia and against human solid tumors xenografted on nude mice.

Table V shows the results of these experiments. For murine L1210 leukemia, results are given in percentage of time of survival compared with untreated controls. Acronycine has only a marginal activity with a T/C of 125 at 200 mg/kg. All the dihydrodiesters exhibit a range of T/C from 200 to 300 at a much smaller dose, demonstrating both a great increase in potency and a broadened spectrum of activity when compared with acronycine itself.

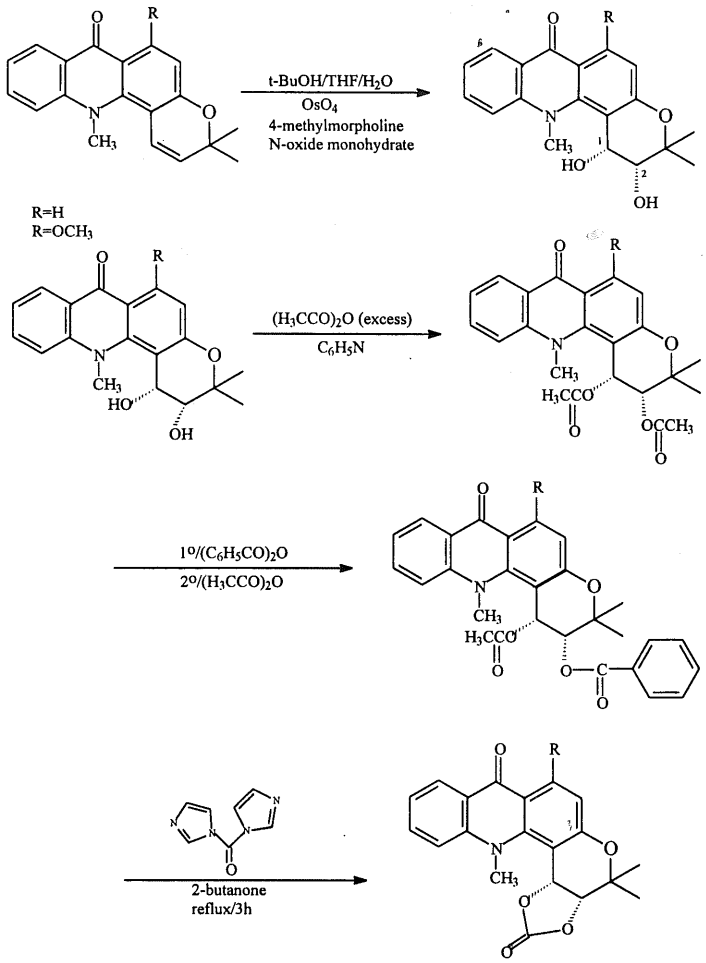
For solid tumors, for example colon carcinoma C38 results are expressed in percentage of tumor growth, three weeks after the graft, followed by two intraperitoneal injections of the drug at days 2 and 9. Acronycine itself is active at 200 mg/kg but it can be observed that the corresponding *cis*-diol diacetate is much more active since a complete inhibition of the tumor growth can be observed for a dose of only 12.5 mg/kg. This latter compound has therefore been selected for preclinical development.

Table V: Antitumor activity of 1,2-dihydroxy-1,2-dihydroacronycine diesters



R_1	R_2	T/C P388	T/C C38 MEDIAN
Acronycine		125 200 mg/kg	4 200 mg/kg
$OCOCH_3$	$OCOCH_3$	289 25 mg/kg	0 12.5 mg/kg
		269 50 mg/kg	68 6 mg/kg
OH	$OCOC_6H_5$	258 12.5 mg/kg	18 6 mg/kg
$OCOCH_3$	$OCOC_6H_5$	201 50 mg/kg	13 12.5 mg/kg

Scheme 4 shows the reaction of the diacetoxy derivative with benzyl mercaptan, used here as a model for cysteine and/or glutathione for solubility reasons. The isolation of both *cis* and *trans* reaction products from the reaction mixture is, of course, in full agreement with our hypothesis. In order to ensure the mechanism of action of our new derivatives at the molecular level, we have reacted 1,2-dihydroxy-1,2-dihydroacronycine diesters with various nucleophilic agents.



Scheme 3. Synthesis of 1,2-dihydroxy-1,2-dihydroacronycine and 1,2-dihydroxy-1,2-dihydro-6-demethoxyacronycine diesters

In conclusion, this paper discusses briefly an example of a chemotaxonomic program and how the study of the structures of natural compounds could stimulate the imagination and the synthetic research in a series of active compounds thus leading to new candidates for cancer chemotherapy.

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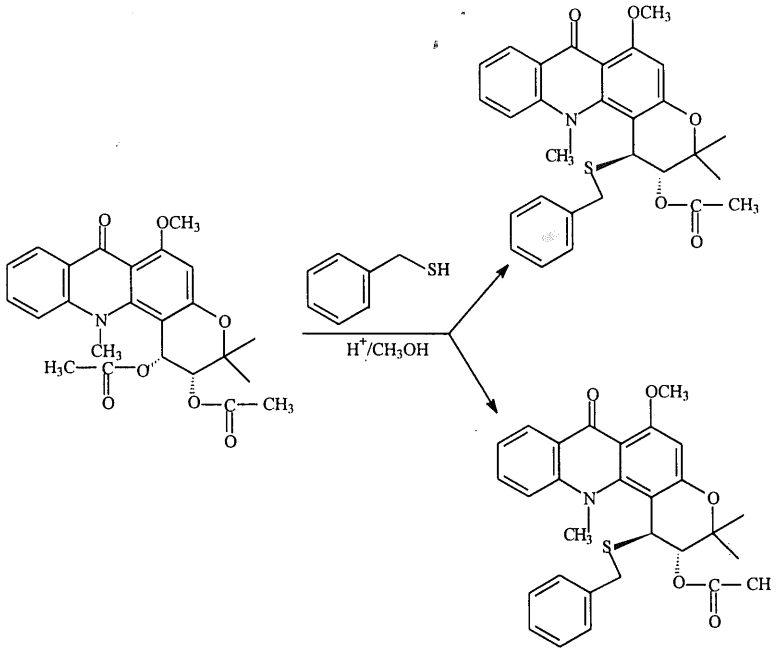

Scheme 4. Molecular mechanism of action of*cis*-1, 2-diacetoxy-1, 2-dihydroacronycine

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