

A. I. Yunusov, N. D. Adbullaev, Sh. Z. Kasymov,  
G. P. Sidiyakin, and M. R. Yagudaev

UDC 547.913

We have previously [1] reported the isolation from the total lactones of a chloroform extract of *Tanacetum pseudoachillea* C. Winkl. [2] of a new sesquiterpene lactone tanachin (I) with the composition  $C_{15}H_{20}O_4$ . In the present paper we give a complete description of the method of determining its structure.

The assignment of the signals in the PMR spectrum of (I) and the determination of the values of  $\delta$  and  $J$  (Fig. 1) were performed by multifrequency resonance. The PMR spectrum of (I) in deuteropyridine solution (see Fig. 1a) shows a clearly separated three proton singlet at 1.49 ppm and a one-proton doublet with broadened components at 5.30 ppm. From their chemical shifts (CSs), they are characteristic for a methyl group and a methine proton, respectively, on a double bond, i.e.,  $CH_3$ -15 and H-5. In actual fact, on irradiation with a strong radiofrequency field of the protons of the methyl group, the components of the signal from H-5 contracted considerably and their intensity rose, which shows the presence of a weak long-range spin-spin coupling (SSC) between them.

At 6.14 and 6.74 ppm there are two one-proton doublet signals, the CSs of which depend fundamentally on the temperature of the sample under investigation. In view of this factor, and also the doublet nature of the splitting and the fact that they are absent from the PMR spectrum of the diacetyl derivative of tanachin (II), the signals observed can be ascribed to the protons of two secondary hydroxy groups (OH-1 and OH-6) present in the molecule of (I). This is also shown by absorption bands in the IR spectrum of (I) (Fig. 2) at 3390 and 3480  $cm^{-1}$  characterizing OH groups. Below we give details of the PMR spectra of tanachin (deuteropyridine, 0 - HMDS):

Protons	$\delta$ , ppm, multiplicity	$J$ , Hz
H-1, H-8	3,94-4,25 m	
2H-2, 2H-3	1,72-2,29 m	
H-5	5,30 br.d	$^3J_{5,6} = 9,8$
H-6	4,42 sex	$^3J_{6,5} = 9,8; ^3J_{6,7} = 9,5$ $^3J_{6, OH} = 3,7$
H-7, H-9	~3,02 m, br.d	$^2J_{9,9'} = -14,1; ^3J_{9,8} < 1$
H'-9	2,50 q	$^2J_{9',9} = -14,1; ^3J_{9',8} = 9,1$
H-13	6,37 q	$^2J_{13,13'} = -1,9; ^3J_{13,7} = 3,3$
H'-13	6,44 q	$^2J_{13',13} = -1,9; ^3J_{13',7} = 2,8$
H-14, H'-14	4,98; 5,07 br.s	
$CH_3$ -15	1,49 br.s	
OH-1	6,14 d	$^3J_{OH,1} = 5,0$
OH-6	6,74 d	$^3J_{OH,6} = 3,7$

Abbreviations adopted: br. s) broadened singlet; br. d) broadened doublet; sex) sextet; q) quartet; m) multiplet.

The chemical shifts of the protons of an exocyclic methylene group conjugated with the carbonyl of the  $\gamma$ -lactone ring are similar, and their signals appear in the form of a two-proton multiplet with centers at 6.37 and 6.44 ppm. When their allyl coupling with H-7 is

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from *Khimiya Prirodnikh Soedinenii*, No. 4, pp. 462-467, July-August, 1976. Original article submitted January 28, 1975.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$7.50.

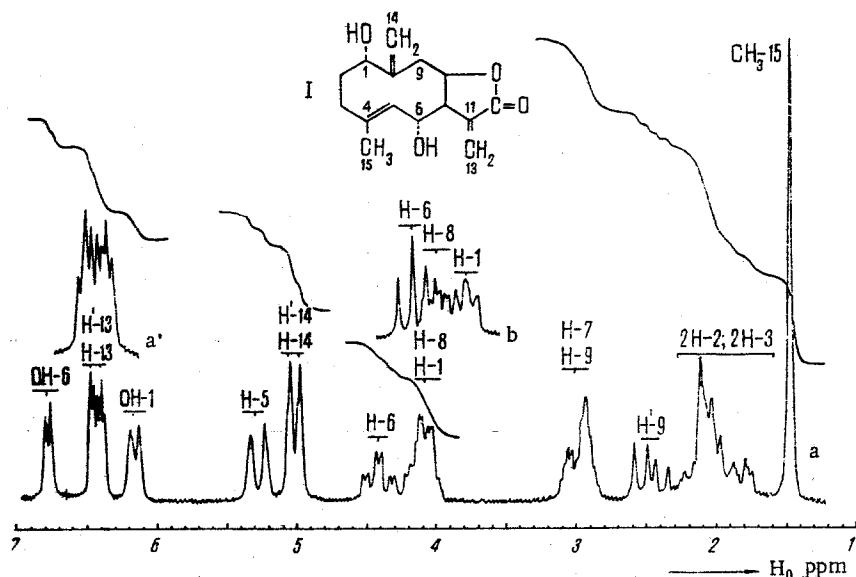


Fig. 1. PMR spectrum of tanachin in  $C_5D_5N$  solution (a, a') and in  $CD_3OD$  solution (b).

suppressed, the multiplet from these protons simplifies to two doublets. The H-7 proton is represented by a signal at ~3.02 ppm which overlaps the broadened doublet from one of the methylene protons in position 9.

When (I) was hydrogenated in the presence of platinum or Pd/C catalysts in ethanol, a dihydro derivative (III) with the composition  $C_{15}H_{22}O_4$  and mp 172-173°C was formed. In this process the exocyclic methylene group in the lactone ring was affected, since the PMR spectrum of (III) did not have the signals of this group, and a three-proton doublet appeared at 1.52 ppm.

One of the protons geminal to a hydroxy group, namely, H-6 is represented by a sextet signal at 4.42 ppm. A study of the double-resonance spectra showed that the nature of the splitting of this signal is due to the interaction of the H-6 proton with three vicinal protons — H-5, H-7, and OH-6 (see above). The establishment of an interrelationship between these protons shows that one of the secondary hydroxy groups is located in position 6. This is also shown by another fact. It is known that if there is a hydroxy group in the  $\beta$  position to an exocyclic methylene of a  $\gamma$ -lactone ring, the difference in the CSs of the protons of the exocyclic methylene must be less than 0.3 ppm [3]. As can be seen from the figures given above, for (I) this difference is 0.07 ppm.

In the PMR spectrum of (I) in deuteropyridine solution, the signals of the lactone proton and of the second hemihydroxyl proton overlap one another. On passing to solution in deuteromethanol, the signals in this part of the spectrum diverge appreciably and their fine structure appears clearly. These changes are shown in Fig. 1b, where the partial overlapping by the triplet from H-6 of the multiplet structure of the signals of the H-8 lactone proton can be seen. This is due to the SSC between H-8 and H-7 with  $^3J = 6.2$  Hz and with two adjacent methylene protons with  $^3J = 2.7$  and 9.2 Hz, the signals from which are located at ~3.02 and 2.50 ppm in the form of a poorly resolved doublet and a quartet, respectively. The latter shows that these methylene protons interact vicinally with only the lactone proton. Consequently, if in the PMR spectrum of (I) there is a singlet from only one methyl group a second exocyclic methylene group, to which a pair of broadened signals at 4.98 and 5.07 ppm correspond, may be located in position 10.

As shown in Fig. 1b, the signal from H-1 of the second hemihydroxyl proton has a triplet nature with  $^3J_{\Sigma} = 14.6$  Hz (in this case, the coupling constant with the protons of the hydroxy group is absent because of the replacement of OH by OD). This means that the second hydroxy group may be present in the vicinal position to the methyl or the exocyclic methylene group and interact with the methyl protons. A group of signals with an intensity of four proton units in the 1.72-2.29 ppm region corresponds to the two methylene groups the protons of which interact with the hemihydroxyl proton under consideration.

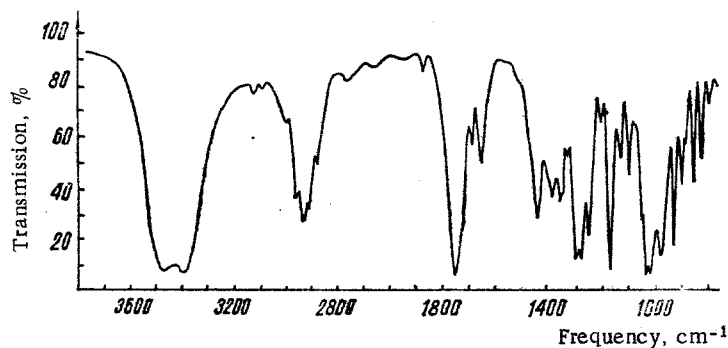
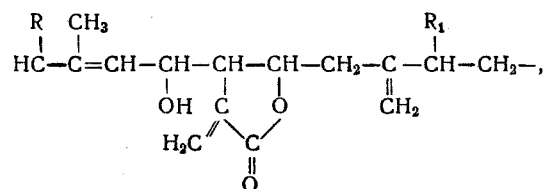


Fig. 2. IR spectrum of tanachin.

Thus, the results of a study of the PMR spectra permit the molecule of (I) to be represented in the following expanded form:



where  $\text{R} = \text{H}$ ,  $\text{R}_1 = \text{OH}$ , or  $\text{R} = \text{OH}$ ,  $\text{R}_1 = \text{H}$ .

Taking into account the sequence of positions of the functional groups given and the composition, it is possible to establish for tanachin a germacrane lactone of linear structure (I).

The hydroxy groups are oriented equatorially, since for H-1 and H-6  $^3J_\Sigma = 14.6$  and  $19.3$  Hz, respectively.

The position of the second hydroxy group (of the two possible ones) was determined by means of a paramagnetic shift reagent (PSR) —  $\text{Eu}(\text{FOD})_3$ . In these circumstances, the lactone that we are considering belongs to a group of polyfunctional oxygen-containing compounds possessing several centers of complex-formation with the PSR. In this case, if the capacity of the functional groups for associating with the PSR that has been shown for monofunctional oxygen compounds [4] is taken into account, a relatively strong complex must be formed by the oxygen atoms of the hydroxy groups and a weaker one by the carbonyl group. An analysis of the structure of tanachin with the aid of Dreiding models has shown that the OH-6 group is spatially less accessible for the formation of coordination bonds with the PSR than the second OH group in position 1 or 3.

In a study of the PMR spectra of (I) in  $\text{CDCl}_3$  solution with the addition of  $\text{Eu}(\text{FOD})_3$ , we obtained the following values of  $\Delta\text{Eu}^*$ : 9.7, 1.1, 3.0, and 4.0 ppm for H-1,  $\text{CH}_3$ -15, H-14, and H'-14, respectively. It can be seen from this that in relation to the  $\text{CH}_3$ -15 the signals from H-14 and H'-14 undergo a paramagnetic shift approximately twice as great. On this basis, we came to the conclusion that the second hydroxy group occupies position 1.

Thus, tanachin has the structure of 1,6-dihydroxygermacr-4,10(14),11(13)-trien-8,12-olide.

#### EXPERIMENTAL

The IR spectra were taken on a UR-20 instrument (tablets with KBr), the PMR spectra on a JNM-4H-100 spectrometer, and the mass spectra on an MKh-1303 instrument. The revealing agent was a 1% solution of vanillin in concentrated sulfuric acid.

Isolation of Tanachin (I). A solution of 185 g of the resin [6] in 30% ethanol was chromatographed on a column of silica gel. Elution was performed with benzene-acetone in various ratios. The fractions were monitored by thin-layer chromatography on Silufol in the benzene-ethanol (17:3) system. The fractions obtained on elution with benzene-acetone

\*We used  $\text{Eu}(\text{FOD})_3$  synthesized in the Perm Pharmaceutical Institute.  $\Delta\text{Eu} = \delta_{\text{Eu}(\text{FOD})_3}^{n=1} - \delta$  of the solvent, where  $n$  is the ratio of the molar concentrations of  $\text{Eu}(\text{FOD})_3$  and the substance [5].

(9:1) yielded another 10 g of tanapsin. The eluates containing 20% of acetone deposited 1.2 g of tanachin with mp 158-159°C (ethyl acetate),  $[\alpha]_D^{23} + 34.8^\circ$  (c 1.84; methanol) mol. wt. 264 (mass spectrometrically),  $R_f$  0.45 (colored crimson by the revealing agent).

Diacetyltanachin (II). A solution of 0.25 g of tanachin in 2 ml of acetic anhydride was treated with 3 ml of pyridine and the mixture was left at room temperature for 8 h. Then it was evaporated in vacuum. The resinous residue was passed through a column of silica gel. The product was eluted with petroleum ether-benzene (1:1). After the solvent had been distilled off and the residue had been recrystallized from benzene-hexane, 0.17 g of the diacetyl derivative with mp 116° and mol. wt. 348 (mass spectrometrically) was obtained. IR spectrum:  $\nu_{\max}$  1780, 1750-1730, 1685, 1665, 1250-1230  $\text{cm}^{-1}$ .

Dihydrotanachin (III). Tanachin (0.2 g) in solution in 15 ml of ethanol was hydrogenated in the presence of 5% Pd/C (0.1 g) for an hour. The catalyst was separated off, and evaporation of the solution gave 0.11 g of dihydrotanachin with mp 172-173°C (benzene-ether), mol. wt. 266 (mass spectrometrically). IR spectrum:  $\nu_{\max}$  3340-3400, 1775, 1670  $\text{cm}^{-1}$ .

#### SUMMARY

1. The new sesquiterpene lactone tanachin (I) has been isolated from a chloroform extract of *Tanacetum pseudoachillea* C. Winkl.

2. With the aid of double and triple resonances, the chemical shifts and coupling constants of the protons of (I) have been determined. On the basis of these results and by the application of a paramagnetic shift reagent  $-\text{Eu}(\text{FOD})_3$  - the structure of 1,6-dihydroxygermacr-4,10(14),11(13)-trien-8,12-olide has been established for tanachin. The orientations of the hydroxy groups have been determined.

#### LITERATURE CITED

1. A. I. Yunusov, N. D. Abdullaev, Sh. Z. Kasymov, and G. P. Sidyakin, *Khim. Prirodn. Soedin.*, 263 (1976).
2. A. I. Yunusov, Sh. Z. Kasymov, and G. P. Sidyakin, *Khim. Prirodn. Soedin.*, 261 (1976).
3. H. Yoshioka et al., *Tetrahedron*, **27**, No. 15, 3317 (1971).
4. J. K. Sanders and D. N. Williams, *J. Am. Chem. Soc.*, **93**, No. 3, 641 (1971).
5. P. V. Demarco et al., *J. Am. Chem. Soc.*, **92**, No. 19, 5734 (1970).
6. A. I. Yunusov, Sh. Z. Kasymov, and G. P. Sidyakin, *Khim. Prirodn. Soedin.*, 309 (1976).