Phytochemistry, 1969, Vol. 8, pp. 2009 to 2012. Pergamon Press. Printed in England.

SESQUITERPENE LACTONES OF *ARTEMISIA*. RIDENTIN*

M. A. IRWIN, K. H. LEE, R. F. SIMPSON and T. A. GEISSMAN

Department of Chemistry, University of California, Los Angeles 90024

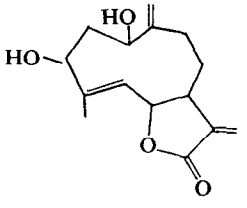
(*Received* 19 *April* 1969)

Abstract—Ridentin, a new germacranolide, has been isolated from several species of *Artemisia* of the Tridentatae complex. The structural relationship of ridentin to other lactones of the complex suggests its position as a precursor in the biosynthetic pathway to the guaianolides of the genus.

RESULTS AND DISCUSSION

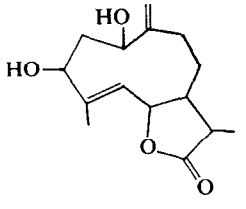
Artemisia species of the Tridentatae group of the Section *Serphidium* (family—Compositae) have been found to contain a large number of sesquiterpene lactones, most of them guaianolides, along with a number of santanolides.[1] A lactone common to a number of Tridentatae (*A. tridentata* Nutt., ssp. *tridentata* and ssp. *parishii* (Gray) H. and C.; *A. cana* Pursh. ssp. *cana; A. tripartita* Gray ssp. *rupicola* (Beetle)), although present in very small amount only, is ridentin, a highly polar (low R_f on TLC) compound, m.p. 215–218° dec.; $[\alpha]_D^{28} -113°$; $C_{15}H_{20}O_4$. Ridentin is an α-methylene-γ-lactone, as shown by its i.r. and u.v. absorption spectra and by the appearance of the pair of low field doublets in the NMR spectrum characteristic of the $=CH_2$ grouping of the lactone.

Ridentin (I) can be reduced with $NaBH_4$ to dihydroridentin (II) and by catalytic hydrogenation to hexahydroridentin (III). These observations, coupled with its composition, show that ridentin is a germacranolide. The presence of two secondary hydroxyl groups is shown by the formation of ridentin diacetate (XI) which, although it could not be obtained as a crystalline material, was purified by chromatography and found to be homogeneous by TLC. Its NMR spectrum was in accord with the structure XI.



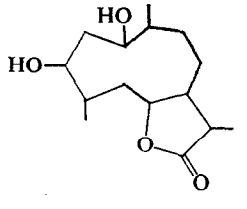
(I)

(XI) (OAc for OH)



(II)

(IV) (OAc for OH)



(III)

(XII) (OAc for OH)

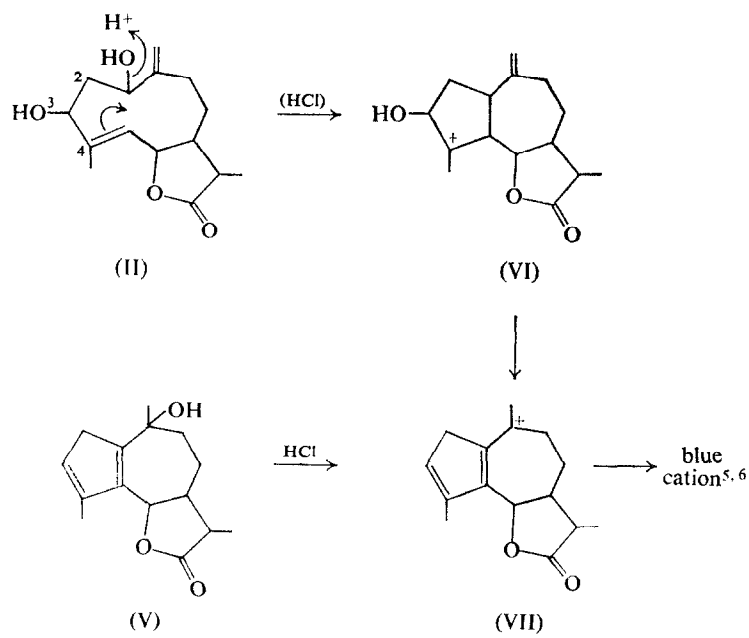
The NMR spectrum of ridentin shows the protons of the exocyclic methylene group (C-13) as a pair of doublets at δ 6·22 and 5·41 (each 1 H, $J=3$ Hz). Ridentin contains but one methyl group, seen as a singlet, slightly split by allylic coupling, ($J=1$ Hz) at δ 1·93 (3 H),

* Contribution No. 2396 from the Department of Chemistry, U.C.L.A.

[1] M. A. IRWIN and T. A. GEISSMAN, studies in progress; to be described in forthcoming publications.

and an exocyclic methylene group which appears as two broadened singlets at δ 4·93 and 5·32 (each 1 H). The hydrogen atoms of the CH—O groupings of the lactone grouping and the two secondary hydroxyl groups were seen as a complex group (total 3 H) between δ 4·18 and 4·77. That the lower of these (4·18) represents the proton of the lactone grouping is revealed by the NMR spectrum of dihydroridentin diacetate (IV), in which a one-proton triplet at 4·41 is now distinct from signals for the protons of the —CHOAc groupings, which have moved downfield to δ 5·02 and 5·27 (each 1 H, doublets of doublets).

The most conspicuous feature of the behavior of ridentin and dihydroridentin is the intense blue color that is formed when their solutions in ethanol are treated with conc. HCl. This property not only resembles that of artabsin (V), which undergoes the same color change, but the acid solutions derived from dihydroridentin and artabsin are nearly identical to visual observation and show absorption maxima at 595 and about 600 nm, respectively.* This behavior under the agency of strong acid has been found to be a valuable diagnostic test for estimating the gross structural types of sesquiterpene lactones.† Its application to the question of the structure of ridentin is that it is a strong indication that ridentin and artabsin are readily converted to a common intermediate; and, because of the fact that the color development occurs rapidly and under mild conditions in both cases, it imposes useful limitations upon the number of possible structures that can be assigned to ridentin. The following formulation, showing the structures (II) assigned to dihydroridentin, and an initial acid-catalyzed ring-closure to an intermediate (VI) indicates the relationship to artabsin as demonstrated by the formation of the same (blue) chromophore from each:

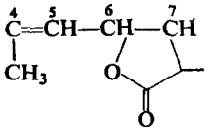


* The substantially identical visual colors and the near identity of the absorption maxima indicate that the chromophores are the same. This color reaction is now under investigation, and it has been observed that the exact position of the absorption maximum is affected slightly by changes in the acid–ethanol ratio used in the test and in the solutions diluted for spectral measurement.

† The nature of these color reactions will be described in a forthcoming paper; certain of the conclusions that have been reached will be assumed for the purposes of the present discussion.

Alternative positions for the two double bonds and the two hydroxyl groups provide less satisfactory rationalizations of the behavior of dehydroridentin in this reaction.

The structural features shown in II are established by further evidence of the NMR spectrum. The relatively high field position (δ 4·4; about 4·2 in ridentin) for the lactonic proton (C-6) and the lower-field positions (δ 5·02, 5·27) for the C*H*OAc protons in dihydroridentin diacetate (IV) indicate that the two hydroxyl groups are allylically disposed with respect to the two double bonds. One of the hydroxyl groups is placed at C-1 because of the ready conversion of dihydroridentin* into the artabsin chromophore (cf. II → VI). The second hydroxyl group is located at C-3 for the following reasons. The NMR spectrum of dihydroridentin diacetate (IV) shows the signal for the single protons at C-1 and C-3 as double doublets at δ 5·02 and 5·07; for the vinyl proton at C-5 as a doublet at δ 5·43 ($J=10$ Hz); and for the proton at C-6 (lactone —C*H*—O—) as a well-defined triplet at δ 4·40 ($J=10$, 10 Hz). The coupling of the vinyl proton at C-5 with the lactonic proton at C-6 was suggested by the identity of the coupling constants, and was demonstrated beyond doubt by double irradiation experiments. When the C-6 proton at δ 4·40 was irradiated, the C-5 proton signal collapsed to a singlet, broadened by allylic coupling with the C-4 methyl group. Irradiation of the C-5 proton at δ 5·43 caused the signal for the lactonic proton at C-6 to collapse to a doublet with $J=10$ Hz. These observations establish the relationship of the protons in the C-5/6/7 system as the following



and, further, show the *trans* fusion of the lactone ring at C-6/7. Finally, the near identity in the coupling patterns of the C-3 and C-5 protons of the —C*H*OAc groupings, and the similarity of their chemical shifts indicates that they are comparably situated, a condition adequately met by their disposition as shown in IV.

Dihydroridentin is also present in the plant examined. Although it was not obtained from the plant in pure form, its presence in the ridentin isolated was shown by the appearance of a small peak at *m/e* 266 ($C_{15}H_{22}O_4$) in the mass spectrum of incompletely purified ridentin, and by the appearance of a small but definite signal (δ 1·22, doublet, $J=6·5$ Hz) for the C-11 methyl group in the NMR spectrum of the latter. Pure dihydroridentin was obtained when the natural ridentin–dihydroridentin mixture was reduced with $NaBH_4$.

The structure of ridentin bears a suggestive relationship to that of isophotosantonic lactone, for a ring closure of ridentin similar to that shown above (cf. II → VI) provides a 3-oxygenated guaianolide that is but a step removed from isophotosantonic lactone. The latter compound has indeed been isolated from *A. tridentata* ssp. *parishii*.[2]

EXPERIMENTAL

Ridentin (I)

Ridentin was isolated as a minor constituent of the more polar fractions during repeated chromatography of extracts of the *Artemisia* species mentioned in the foregoing discussion. Systematic combination and re-chromatography of fractions that were found (by TLC) to contain it led to its eventual isolation.

* Ridentin itself gives the same color reaction, but the blue color is unstable, in contrast to the dihydro compound. The color produced by the latter is, as is that formed from artabsin, quite stable, persisting with no obvious visual change for many hours.

[2] M. A. IRWIN and T. A. GEISSMAN, to be published.

Ridentin forms colorless crystals with m.p. 215–218° dec. It had $[\alpha]_D^{25}$ −113° (*c* 0·46, MeOH). Its mass spectrum showed the molecular ion at *m/e* 264 (along with a smaller peak at *m/e* 266) and prominent peaks at *m/e* 246 (M-18) and 228 (M-36). The i.r. spectrum showed peaks at 3300 (broad, OH); 1765, 1650 (exocyclic methylene α,β-unsaturated-γ-lactone); and 1670 (double bond). The u.v. spectrum showed end absorption with $\epsilon = 13{,}700$ at 210 nm. The salient features of the NMR spectrum have been described above. (Found: C, 68·28; H, 7·69. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63%.)

Dihydroridentin (II)

To a solution of 1·06 g of ridentin in 60 ml of methanol was added an excess of $NaBH_4$. The solution was allowed to stand at room temperature for 3·5 hr, then evaporated *in vacuo*, diluted with water and extracted with $CHCl_3$. The washed and dried (Na_2SO_4) $CHCl_3$ solution was evaporated to dryness, yielding a white solid, m.p. 190°. Recrystallized from ethyl acetate, the compound formed colorless needles, m.p. 193–194° (237 mg). (Found: C, 67·40; H, 8·04. Calc. for $C_{15}H_{22}O_4$: C, 67·64; H, 8·33%.)

The NMR spectrum showed the absence of the low-field signals for the protons of the exocyclic methylene group of the lactone, and the appearance of a three-proton doublet for the C-11 methyl group (C-13) of the dihydro compound.

Hexahydroridentin (III)

A solution of 50 mg of dihydroridentin in 10 ml of methanol was hydrogenated in the presence of Adams' catalyst (20 mg) at ordinary pressure. After 4·5 hr the filtered solution was evaporated and the oily residue chromatographed over silica gel. Elution with acetone yielded fractions (combined by inspection of TLC plates) from which there was obtained a residual oil which crystallized when rubbed with ether. Recrystallized from CH_2Cl_2–ether, the compound formed fine, colorless needles, m.p. 118–120°. (Found: C, 66·60; H, 9·63. Calc. for $C_{15}H_{26}O_4$: C, 66·63; H, 9·69%.)

The NMR spectrum showed three three-proton doublets at δ 1·07, 1·12 and 1·20 (all with $J = ca.$ 7 Hz), and the complete absence of signals in the vinyl region below δ 4·45, at which frequency appeared the signal (1 H, mult.) for the C-6 proton of the lactone groupings.

Acetates

The diacetates (XI, IV and XII, respectively) of ridentin, dihydroridentin and hexahydroridentin were prepared with the use of acetic anhydride-pyridine. None was obtained as a crystalline compound, but each was purified to homogeneity on TLC. The NMR spectra of the purified diacetates were in every case in accord with expectation, showing the two signals for the methyl groups of—$COCH_3$ at about δ 2·0–2·1, and the downfield shift (compared with the unacetylated compounds) of the protons of the —*CH*—OH(Ac) groups.

The most revealing features of the NMR spectra of ridentin and ridentin acetate were the positions of the signals for the vinyl methyl groups (at C-4) of these compounds. In ridentin, the C-4 methyl group appears as a three-proton signal at δ 1·93. In the acetate (XI), this methyl group is seen at δ 1·69. This shift shows that the vinyl methyl group is strongly influenced by the acetoxy group in the acetate, an observation in accord with the placement of the hydroxyl group at the adjacent position 3.* The corresponding shift is seen in the case of dihydroridentin (C-4 CH_3, δ 1·91) and its acetate (IV) (C-4 CH_3, δ 1·72).

Color Reaction

When a few drops of conc. HCl† are added to a solution of a few mg of artabsin or dihydroridentin in ethanol, the solution assumes a deep blue color. Artabsin gives the color instantly in the cold, dihydroridentin after gentle warming. When ether and an excess of water are added to the blue solution, the ether layer that separates is yellow. Removal of the clear yellow ether layer and addition to it of conc. HCl regenerates the blue color. The acidic, blue solutions were diluted to a suitable concentration for measurement of the absorption spectra.

Acknowledgements—This study was supported by a U.S. Public Health Service research Grant GM-14240-03. Part of the costs of the NMR and mass spectrometers were borne by grants to the Chemistry Department from the National Science Foundation and E. I. du Pont de Nemours and Company. Analyses are by Miss Heather King. We wish to thank Professor F. A. L. Anet for his valuable assistance in carrying out the decoupling experiments.

* These methyl group signals are broadened ($J = 1$ Hz) by coupling with the vinyl proton at C-4.

† Other strong acids behave similarly: $HClO_4$, HBr, H_2SO_4, BF_3-etherate all give blue to purple-blue colors, all superficially nearly the same to visual observation, but some differing noticeably in the exact cast of the color. Spectral measurements to pursue these differences are in progress.