Pergamon

Tetrahedron Letters, Vol. 35, No. 9, pp. 1385-1388, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(94)E0040-5

SPIROCARACOLITONE ISOLATED FROM A NEW GENUS AND SPECIES, *RUPTILIOCARPON CARACOLITO*. THE FIRST CD SPIRO-TRITERPENOID.

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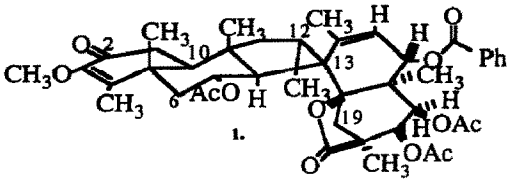
Abstract: The novel spiro-triterpenoid spirocaracolitone, was isolated from a newly described genus and species, *Ruptiliocarpon caracolito* Hammel and Zamora (Lepidobotryaceae), a tree from humid lowland tropical rainforest in Costa Rica. Spectroscopic methods allowed for the elucidation of fragments of the molecule but only X-ray diffraction yielded the definitive structure. Spirocaracolitone has a unique, previously unreported CD spiro friedelin skeleton.

As part of the program aimed at the development of "green insecticides"[1], we have screened extracts from various genera of the Rutales collected in Costa Rica for growth reducing activity against the European corn borer (*Ostrinia nubilalis*).

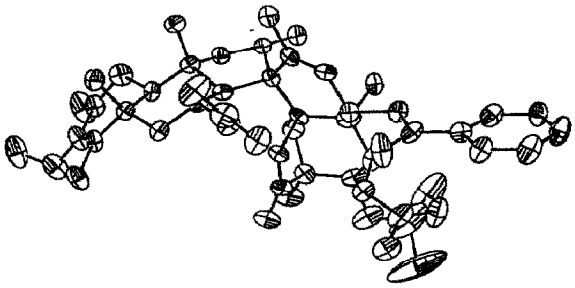
A screen of approximately sixty ethanol extracts of various parts of twenty seven species, with emphasis on the Meliaceae, against European corn borer[2] revealed several active extracts. The most active, with an RGR of 19.54[3], was obtained from the bark of the tree *Ruptiliocarpon caracolito*, an endemic species collected near Golfito and in the Osa peninsula, Costa Rica. Based on similarity of the wood of *R. caracolito* to *Trichlia* ssp.(Meliaceae) and floral similarities, notably the filament tube, of *R. caracolito* and various Meliaceae, this unusual species was first thought to be a member of the Meliaceae.[4] Recent studies suggest its position as a unique American genus and species of the family, Lepidobotryaceae with close affinity to the monotypic African genus *Lepidobotrys*. Bioassay guided fractionation of this bark extract led to the eventual isolation of a highly unusual triterpene **1**, which features a highly oxidized ring A, a C_{30}-C_{18} γ-lactone and a novel spiro junction between rings C and D. To the best of our knowledge this represents the first example of a spiro CD junction in pentacyclic triterpenes.

The air dried bark of *Ruptiliocarpon caracolito* was ground with a Wiley mill, allowed to soak in 95% ethanol at room temperature overnight and the ethanol was filtered off. This extraction process was performed three times. The freeze dried extract was separated into hexane, dichloromethane and water soluble portions

which were bioassayed for activity.[5] The dichloromethane soluble fraction, which contained most of the activity, was subjected to silica gel column chromatography using 2:3 ethyl acetate/hexane to 1:9 methanol/ethyl acetate as the eluents. Further purification of the most active multicomponent fraction by HPLC[6] afforded a major compound with a melting point of 215-218°C , $(\alpha)^{25}_D$= +39.34 (c= 0.0061, CH_2Cl_2) and FAB MS, *m/z* =775.4 $(M + 1)^+$. The structure of spirocaracolitone ($C_{44}H_{54}O_{12}$) was established by nmr and single crystal X-ray[7] as **1**.

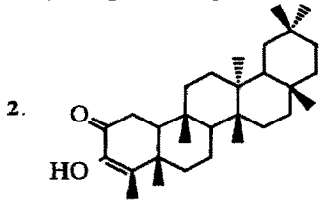


1.



Spirocaracolitone showed infrared absorption at 1780,1750 and 1722 cm^{-1} respectively characteristic of the E ring lactone, the acetate and benzoate ester functionalities, and the α,β-unsaturated ketone in ring A. The unsaturation in ring D occurred at 1674 cm^{-1}. The combination of advanced NMR experiments and the X-ray structure has allowed us to assign all of the proton resonances.[8] An in depth discussion of the assignments in both the proton and carbon NMR's will be reported elsewhere.

The structure of spirocaracolitone is most interesting from a biosynthetic point of view since several of the structural features are rare or have not been describes previously in pentacyclic triterpenoids. There appear to be no examples of such species bearing a C12 methyl group or a spiro CD ring system and it is highly probable that these two features are biogenetically interdependent. Spiro systems in triterpenoids are rare, a recent example which has a spiro BC ring is spirosupinanonediol isolated from *Euphorbia supina*.[9] The oxidation pattern of ring A is also unusual but examples do exist, e.g. 3-hydroxy-friedel-3-en-2-one, **2** isolated from the bark of *Quercus suber*.[10] The presence of the C30-C18 γ-lactone, the C21 and C22 cis diacetates and the C16 allylic benzoate are features which have been individually reported previously[11] but not in combination.



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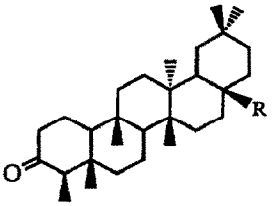
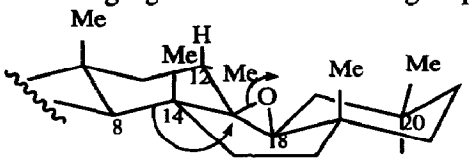


3a. R=CH_3
3b. R=CH_2OH

Compound **1** is a rearranged highly oxidized friedelin derivative. It is interesting to speculate on the genesis of the spiro ring system. This can plausibly be derived from a species such as **2** or **3a** in which generation of a carbocation at C12 is followed by migration of the β-C13 methyl group. The newly created C13 carbocation could either lose H18 or undergo a C8-C14 bond migration to generate the CD spiro ring system and thus the carbon skeleton of spirocaracolitone. Alternatively, the spiro system at C13 could be set up by ring opening of a C13-C18 β−epoxide and migration of the antiperiplanar C8-C14 bond (partial structure **4**). This pathway also results in the formation of a tertiary hydroxyl group at C18 which could readily be displaced by the C30 carboxylate to form the γ-lactone (partial structure **5**). Chromatography of the hexane extract has afforded the friedelin derivative canophyllol, **3b**. Thus *R. caracolito* appears to be a source of compounds belonging to the friedelin group.



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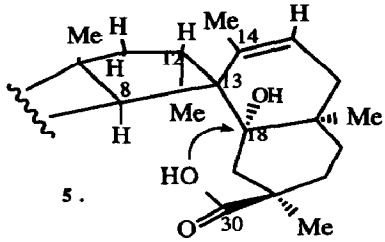

5.

When tested alone against the second instar European corn borer **1** did not fully explain the activity of the active fraction suggesting that the initially observed activity is present in another part of the dichloromethane extract or the activity is a result of synergism with other components. A more complete evaluation of spirocaracolitone's antifeedant activity via a neonate lifecycle study is presently underway. Further investigation of the other components of this active fraction is being actively pursued to isolate other related triterpenoid structures which could aid in the chemotaxanomic placement of *R. caracolito* and possibly help determine the biogenesis of spirocaracolitone.

ACKNOWLEDGEMENTS: Funding was provided by a grant from NSERC Canada and Forestry Canada's Greenplan initiative. We would like to thank Prof. J. Connolly (Glasgow) for comments concerning the novelty of spirocaracolitone.

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1. Botanical insecticides with non neurotoxic modes of action, possessing growth reducing or antifeedant activity.
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3. RGR = relative growth of larvae as % of the control.

4. Hammel, B.E. and Zamora, N.A. *Novon* **1993**, (in press)
5. The hexane soluble fraction was obtained by extracting a solubilized portion of the extract in ethanol/water 1:1 three times with hexane. Removal of the fraction. The water soluble fraction was obtained by freeze drying the remaining aqueous layer.
6. Preparative HPLC equipped with a Techsphere 5µm ODS column (25cm X 10mm), a variable UV detector set at 254 nm and running isocratically with 60/40% acetonitrile/ water (flowrate= 14 mL/min) was effective in seperating the minor components from the one major peak. Spirocaracolitone had a retention time of 20-22 min. Dry bark(100g) yielded approximately 33 mg of a white solid **1**.
7. Crystallographic data for Spirocaracolitone : $C_{44}H_{54}O_{12}$ .CH_3COCH_3. CH_3OH. H_2O FW= 897.07 , monoclinic, space group P 21. Lattice parameters a=10.853(7), b=16.713(7), c=13.071(5), β=96.15(4) , Z=2, D_{calcd}=1.264 g/cm^3. The data was collected at -140° C using the omega-2theta scan technique to a maximun 2theta value of 46.9 degrees. Crystal dimensions 0.2, 0.1, 0.2 mm. Intensity data were recorded on a Rigaku diffractometer with Mo Kalpha radiation. The crystal structure was solved by direct methods. All the atoms were refined anisotropically except the hydrogen which were calculated. All the refinements were made using the unobserved data to increase the ratio of reflections per parameters. The final cycle of full matrix least-squares refinement was based on 2969 observed reflections (I>2.5 sigma(I)) and 538 variable parameters, giving a R=0.102 (Rw=0.092). Weights based on counting statistics were used. The maximun and minimum peaks on the final differences Fourier map corresponded to 0.670 and- 0.430 e/a3, respectively. All calculations were performed using the NRCVAX crystallographic software package.
8. ^{1}H NMR($CDCl_3$, 500 MHz Bruker) MeO: 3.61, Methyl and acetate methyl's: 1.18, 1.22, 1.23, 1.23(d), 1.47, 1.83, 1.87(d), 1.91, 1.94, 2.28 CH_2's: C(1)H_2- 2.59(dd, J=17.2, 14.3 Hz) and 2.34(dd, J=17.2, 3.4 Hz), C(6)H_2- 2.11(dd, J=12.2, 3.2 Hz) and 1.54-1.45(m), C(11)H_2- 1.35-1.55(m), C(19)H_2- 2.43(d, J=12.9 Hz) and 2.62(d) CH's: C(7)H- 5.31 (dt, J=3.2, 10.9 Hz), C(8)H-2.3-2.7, C(10)H- 1.85-1.80(m), C(12)H- 2.92(m), C(15)H- 5.45(bs), C(16)H- 6.02(m), C(21)H and C(22)H-(dd, J= 4.4, 5.21 Hz), Benzoate: 7.88-7.39. ^{13}C NMR($CDCl_3$, 300 MHz Varian): 11.4, 16.3, 17.9, 18.5, 19.4, 20.3, 20.4, 21.3, 21.4, 23.9, 37.2, 40.0, 41.8, 42.4, 43.8, 44.2, 45.9, 47.1, 50.4, 55.9, 57.5, 59.7, 61.6, 66.8, 69.9, 72.1, 72.7, 90.5, 125.4, 128.4, 129.4, 129.8, 133.2, 166.0, 169.8, 170.1, 171.6, 193.9.
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(*Received in USA* 13 *October* 1993; *revised* 15 *December* 1993; *accepted* 22 *December* 1993)