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# ISOLATION, STRUCTURE DETERMINATION, AND ABSOLUTE CONFIGURATION OF BARBATUSOL, A NEW BIOACTIVE DITERPENE WITH A REARRANGED ABIETANE SKELETON FROM THE LABIATE *COLEUS BARBATUS*

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**Abstract**—Barbatusol, isolated from the Labiate *Coleus barbatus* Bentham, is shown to be a new phenolic diterpene with a rearranged abietane skeleton. Absolute configuration is established by hemisynthesis of barbatusol dimethyl-ether from the known carnosol. Barbatusol is endowed of biological activity.

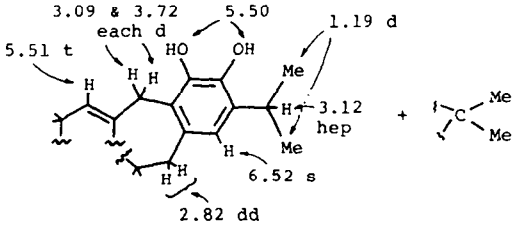
Known as "boldo" in Brazil, the Labiate *Coleus barbatus* Bentham is largely used in popular medicine against liver fatigue and intestinal disorders. Several abietane diterpenes have been isolated from the leaves of the plant grown in Brazil[1] whilst the leaves of *C. barbatus* of Kenya afforded highly unsaturated rearranged abietanes[2] and the roots of the Indian *C forskolii* (syn of *C. barbatus*) furnished polyhydroxylated labdane diterpenes,[3] two of them, forskoline and colenol, exhibiting potent antihypertensive activity.[4,5]

This prompted us to reinvestigate the Brazilian *C. barbatus* looking for new cardioactive compounds.[6] In this paper, we wish to report on the isolation, structure elucidation, absolute configuration and partial synthesis of barbatusol (I),[7] (5*S*)-9(10→20)-*abeo*-abieta-1(10), 8,11,13-tetraen-11,12-diol, a new bioactive diterpene obtained from *C. barabatus* collected in the surroundings of Rio de Janeiro.

In a previous communication,[6] we reported that the dichloromethane crude extract of the bark and heartwood of *C. barbatus* showed, at doses of 10 mg/kg (i.v.), a small cardiovascular activity in nembutal anaesthetised rats. Purification of the crude extract was monitored by bioassays and involved gel filtration on Sephadex LH-20 followed by silica gel column chromatography. Besides (+)-ferruginol (II) (yield: 0.07%), we isolated a yellow gummy compound, turning to reddish-brown on standing, for which the name barbatusol[7] (yield: 0.15%) was coined, and which induced at 3 mg/kg (i.v. rats) potent lowering of blood pressure associated to discrete bradycardy.

High resolution mass spectrometry of barbatusol (I) established empirical formula $C_{20}H_{28}O_2$ (300.2089, requires 300.2089). The IR spectrum shows OH absorption ($\nu_{OH}$ 3580 and 3540 $cm^{-1}$). Acetylation of I afforded diacetate III showing that both O atoms are part of OH groups. The phenolic nature of the OH groups was suggested by the absence from the $^{13}C$ NMR spectrum of I of any saturated C substituted by an O atom, and also by the position of the CO vibrations in the IR spectrum of III ($\nu_{C=O}$ 1760 $cm^{-1}$) together with the low field position of both Me signals (2.29 and 2.30 ppm) and the absence of deshielded carbinol protons in the $^1H$ NMR spectrum of diacetate III. The diphenol was further substantiated by the UV spectrum of I ($\lambda_{max}$ 275 nm, $\epsilon$ = 2400 and 210 nm, $\epsilon$ = 18000) also showing that the OH groups are not *para* to each other.[8] The *ortho* relationship between the OH groups was established by $Ag_2O$ oxidation of I into *o*-quinone IV characterized by CO absorptions in IR at 1670 and 1645 $cm^{-1}$ and UV absorptions at 438 ($\epsilon$ = 1000), 270 ($\epsilon$ = 4095) and 227 nm ($\epsilon$ = 8390).[9] The pentasubstituted nature of the aromatic ring depended on the presence in the $^1H$ NMR spectrum of a 1H aromatic broad singlet at 6.52 ppm in I deshielded to 6.92 ppm in III. Four of the seven unsaturations of barbatusol (I) are introduced by the aromatic nucleus. A fifth was found associated with a trisubstituted double bond (IR: 970 $cm^{-1}$; $^1H$ NMR: 1H t at 5.50 ppm J = 3.0 Hz; $^{13}C$ NMR: 140.25 ppm (s) and 120.89 ppm (d)). The two remaining unsaturations were thus attributed to two additional rings. Hence, barbatusol (I) is a tricyclic aromatic diterpene for which an abietane skeleton could be postulated since several abietane diterpenes, including (+)-ferruginol (II), had already been isolated from *C. barbatus*.[1,6] The $^1H$ NMR spectrum of I shows striking similarities with that of pisiferol (V),[10] and was characterized by the presence of only four Me signals, two as superposed doublets (J = 7.0 Hz) at 1.20 ppm and two as singlets at 0.85 and 0.89 ppm. Noteworthy was the absence of the C-20 Me signal, a singlet at *ca* 1.20 ppm in the ferruginol series.[11] The two Me singlets were associated with a *gem*-dimethyl moiety since only one quaternary saturated C was observed in $^{13}C$ NMR (32.01 ppm,s). On the other hand, double irradiation experiments proved that the two Me doublets were part of an isopropyl group whose methine proton shift (3.10 ppm) suggested the *i*-propyl group to be linked to the aromatic ring.[11] This was confirmed by the small upfield shift (0.23 ppm) of the methine proton of the isopropyl group observed, on acetylation, in the $^1H$ NMR spectrum of III. This spectrum also shows that the Me groups of the isopropyl were no longer equivalent (two 3H d J = 7.0 Hz at 1.19 and 1.20 ppm) due to hindered free rotation of the isopropyl group[12] and suggested that the latter is probably flanked by an OH group. The C-7 benzylic methylene of tricyclic aromatic diterpenes[12] was observed as a 2H ill-resolved dd ($W_{1/2}$ = 16 Hz) at 2.82 ppm; the multiplicity of this signal showed that the C-7 methylene is adjacent to another methylene group. Finally, a 2H AB quartet (J = 15 Hz) was observed at 3.09 and 3.70 ppm; the large coupling constant associated to it was found in good agreement with a *gem* coupling.

The low field position of this methylene group suggested it to be adjacent to two deshielding groups, namely the aromatic ring and the trisubstituted double bond. This conclusion was further supported by catalytic hydrogenation of I to the mixture of epimers at C-1 ($VI_{a+b}$) whose $^1H$ NMR spectrum was devoid of the AB signals downfield from 3.00 ppm. Furthermore, the absence of coupling between the olefinic proton and the allylic–benzylic methylene allowed to propose the following sequence: ≀-CH=C(-H)-CH₂-Ø. Indeed, irradiation of the vinylic proton (bt at 5.50 ppm J = 3 Hz) did not affect the AB pattern, but slightly modified the spectrum at 1.90 ppm. Conversely, irradiation at the latter frequency transformed the triplet at 5.50 ppm into a broad singlet, but had no effect on the C-7 methylene signal at 2.82 ppm. Based on the abietane skeleton hypothesis (for the arrangement of the aromatic substituents), fragment "A" best accomodate for all our observations.




A

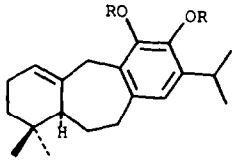
However, the presence of two benzylic methylenes suggested that barbatusol had a rearranged abietane skeleton where the missing C-20 Me group should be included in ring B, by formal migration of the $C_9$–$C_{10}$ bond into the $C_9$–$C_{20}$ position and subsequent loss of one proton at C-1 to give rise to the trisubstituted double bond between C-1 and C-10. Hence, structure I could be proposed for barbatusol. The $^{13}C$ NMR spectra of I and III were found consistent with this proposal.

Final proof of structure I and determination of the absolute configuration of barbatusol came by obtention of barbatusol dimethyl-ether (XII) from carnosol (VII), a diterpene whose absolute configuration had been established by correlation with (+)-ferruginol (II).[12]

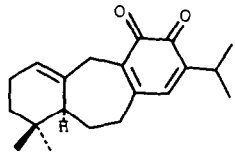
Carnosol (VII) was isolated from the cultured Rosemary (*Rosmarinus officinalis*) by techniques identical to those used for purification of barbatusol. Thus, obtention of barbatusol dimethyl ether (XII) from VII was achieved, after protection of the catechol moiety, by removal of the functionality at C-7; rearrangement of a C-20 hydroxymethylene group, leading to B-ring expantion, furnished the desired derivative XII (Scheme 1).

Under anhydrous conditions, methylation of carnosol (VII), into dimethyl-ether XIV, is known to proceed slowly.[13] When conducted in wet acetone with $K_2CO_3 \cdot 2H_2O$ as catalyst, methylation of the phenol groups is accompanied by opening of the lactone ring and elimination of the O function at C-7 affording compound VIII in one step. These conditions are not sufficient to promote alkaline hydrolysis of the δ-lactone.[12,13] Since carnosol dimethyl-ether (XIV) did not react under similar operating conditions, assistance of the OH group at C-12 could be postulated. Obtention of VIII may thus proceed as proposed in Scheme 2. Carnosol dimethyl-ether (XIV) was also obtained, together with VIII, but in lower yield.

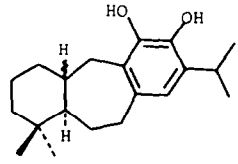
Catalytic hydrogenation of VIII readily furnished IX, a derivative already obtained by total methylation of carnosic acid (XV), but for which only partial chemical data had been reported.[14,15] LAH reduction of the ester IX, inefficient at room temperature, furnished on refluxing the expected hydroxymethylene X in only 60% yield, together with carnosic acid dimethyl-ether (XVI; 23%). Acid XVI is obviously not an intermediate in the reduction of IX into X since the latter was not produced on LAH treatment of XVI in refluxing THF. Strong steric hindrance by the axial β-Me group at C-4 may explain the low yield in X.[16] Treatment of X with TsCl[17] afforded the less polar XI homogeneous in TLC in various solvent systems. The IR spectrum of XI proved the absence of an OH or tosyl group, and was found practically iden-



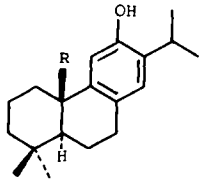

I R = H
III R = Ac
XII R = Me



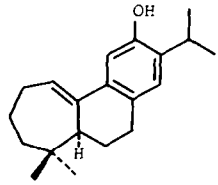

IV



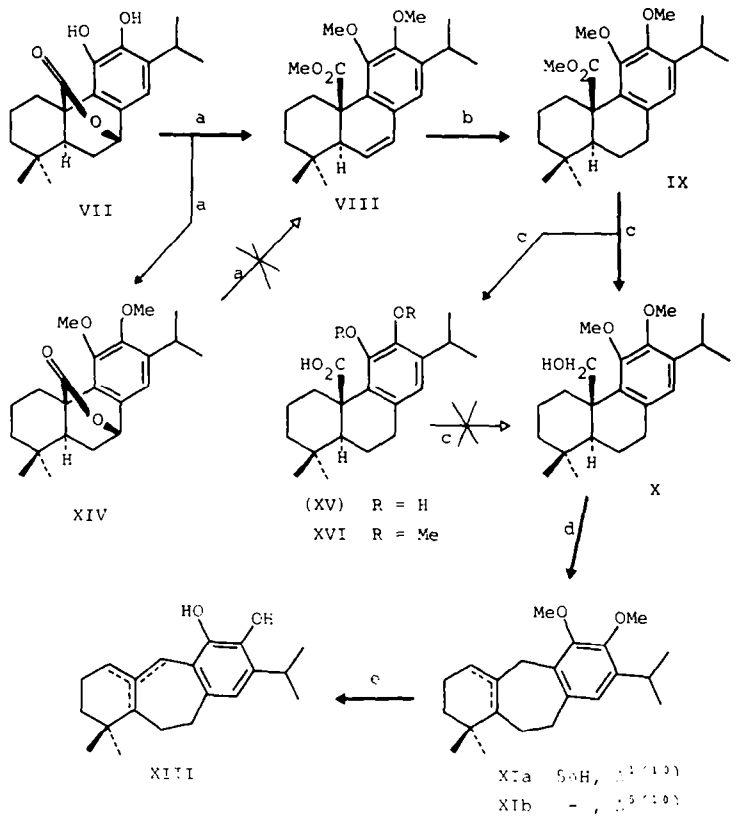

$VI_{a+b}$



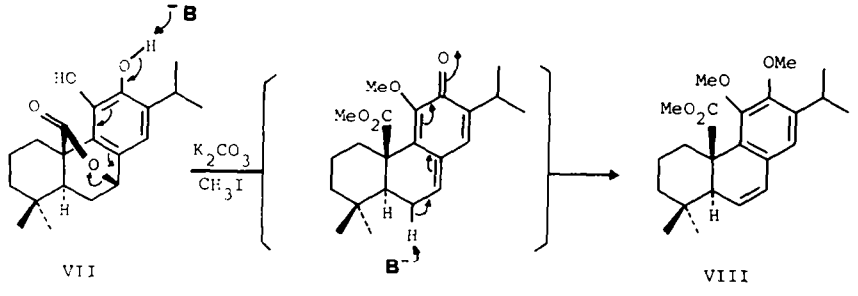

II R = Me
V R = $CH_2OH$




XVII



Scheme 1. (a) $CH_3I$–$K_2CO_3 \cdot 2H_2O$–acetone–room temperature–1 day; (b) $H_2$/10% Pd–C–45 psi–AcOEt–room temperature–3 hr; (c) LAH–dry THF–reflux–3 hr; (d) TsCl excess–dry Py–room temperature –20 hr; (e) $BBr_3$–dry $CH_2Cl_2$–room temperature–10 min.



Scheme 2.

tical to the IR spectrum of barbatusol dimethyl-ether (XII), obtained by direct methylation of barbatusol (I). GLC analysis (3% OV-17), MS and UV data also supported the identity of XI with XII. However, $^1$H NMR showed XI to be a 6:4 mixture of double bond isomers identified as $XI_a$ and $XI_b$. All our attempts to separate both isomers on silver nitrate impregnated silica gel TLC plates failed. The obtention of $XI_a$ from carnosol (VII) proved the correctness of the structure of barbatusol (I).

Optical rotation measurements of barbatusol dimethyl-ether (XII) and of the synthetic material $XI_{a+b}$ showed that both samples were laevorotatory. Since $XI_b$ is optically inactive, this proved barbatusol dimethyl-ether (XII) to have the same 5*S* absolute configuration as $XI_a$, i.e. the H at C-5 is α-oriented. This conclusion could have been anticipated since it is the same absolute configuration as that of (+)-ferruginol (II) isolated from *Coleus barbatus* together with barbatusol (I). Hence barbatusol is: (5*S*)-9(10→20)-*abeo*-abieta-1(10), 8,11,13-tetraen-11,12-diol. It seems reasonable to speculate that the driving force for the hydroxymethylene rearrangement of X into XI, promoting the B-ring expansion, may be seen in the presence of the bulky OMe group at C-11, since TsCl rearrangement of pisiferol (V) led only to A-ring enlargement, affording pisiferin (XVII).[18] Finally, treatment of the mixture $XI_{a+b}$ with $BBr_3$ in dry $CH_2Cl_2$[19] afforded, but in poor yield, the mixture of double bond isomers XIII, where barbatusol (I) is less than 10% abundant as deduced from the $^1$H NMR spectrum of XIII.

Barbatusol (I) is one of the few representatives of the highly unusual 9(10→20)-*abeo*-abietane skeleton, only found in icetexone and romulogarzone, two diterpenes isolated from the Labiate *Salvia ballotaeflora*,[20] and in nilgherron A and B, two bis-diterpenes obtained from another Labiate, *Plectranthus nilgherricus*.[9] As far as we know, no biological activities were reported for these compounds. The structures of icetexone and romulogarzone came from X-ray diffraction analysis and spectral data respectively and the nilgherrons A and B had their absolute configurations proposed on biogenetic considerations only (at least for what concerns C-5 of the *abeo*-abietane moiety). The bioactive fraction we isolated from *Coleus barbatus* contains at least three aromatic diterpenes in addition to the reported (+)-ferruginol and barbatusol. Their structure elucidation and biological activity investigation are under way in our laboratory.

## EXPERIMENTAL

M.ps were determined on a Büchi SMP-20 apparatus or on a Kofler hot stage and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at ambient temp. in $CHCl_3$ solns. IR spectra were recorded in $CHCl_3$ solns with a Perkin–Elmer 735B spectrophotometer or as films with a Perkin–Elmer 137 apparatus. Low resolution mass spectra (MS) were determined on a Micromass MM12F instrument; high resolution MS were determined on a Varian CH 5 DF instrument; intensities of the fragments are expressed as percentages of the base peak (100%). $^1$H NMR spectra were recorded with a Varian XL-100 or with a Varian EM 390 apparatus equipped with the Varian 3930 spin decoupler system; unless otherwise mentionned, spectra were recorded in $CDCl_3$ solns using TMS as internal reference; shifts are expressed in the $\delta$ scale; the following abbreviations are used: b = broad, d = doublet, m = multiplet, t = triplet, s = singlet, q = quadruplet and hep = heptuplet. UV spectra were recorded in MeOH solns on a Varian Techtron 635 or on a Beckman DB-CT grating spectrophotometer coupled to a Linear 261 MM or to a Beckman 10″ recorder. GLC were performed on a Varian Aerograph 2800 apparatus, coupled to a Varian A-25 recorder, and equipped with a FID detector system, on a 7′ × 1/8″ stainless steel column packed with 3% OV-17 on Varaport 30, operating in isotherm mode at 235°. Analytical and preparative TLC were performed on E. Merck Kieselgel $HF_{254+355}$ self made plates, the analytical chromatograms being revealed by UV light ($\lambda_{254}$, Desaga apparatus) and by spraying a 3% phosphomolybdic acid soln or a 0.2% soln of ceric sulfate in 2N $H_2SO_4$ followed by heating 5 min at 150°. Column chromatographies were performed on E. Merck Kieselgel 60 (70–230 mesh). Sephadex LH-20 (Pharmacia, Uppsala) was used for gel premeation chromatographies.

*Isolation of barbatusol* (I) *and* (+)-*ferruginol* (II).

*Coleus barbatus* Bentham was collected in the surroundings of Rio de Janeiro in April 1978. More material was obtained in October 1981, affording identical diterpene composition. Leaves and stems were separated, air-dried and the stems pulverized in a hammer-mill. The stems (600 g) were extracted exhaustively with $CH_2Cl_2$ at room temp. for several days. The combined $CH_2Cl_2$ extracts were evaporated to dryness under reduced pressure to afford a brownish residue (48 g) which was dissolved in MeOH (150 ml). The insoluble material (29g), mainly composed of waxes, was separated by filtration. The filtrate which contain all the biological activity[6] was concentrated to 15 ml; 2 ml aliquots of this soln were applied successively on a 250 ml (total volume bed) bed of Sephadex LH-20 swollen in MeOH and eluted with pure MeOH (5 ml/min). One fraction of 75 ml and 20 fractions of 5 ml were collected, then a final fraction of 100 ml. Fractions 14–20 were found to contain all the biological activity. A second gel filtration of these combined fractions furnished about 5 g of an active fraction[6] composed of at least 5 compounds. Rapid silica gel filtration of the latter cardioactive fraction (eluent: pure $CH_2Cl_2$, then pure AcOEt) yielded pure (+)-II (0.07% from dry plant material) and I (0.15% from dry plant). The other compounds were still obtained as a mixture.

(+)-ferruginol was identified by $[\alpha]$, UV, IR, MS, $^1$H and $^{13}$C NMR identical to reported data.[11,12]

*Barbatusol* (I): amorphous; $[\alpha]_D = -102.5$ ($c = 1.88$ in $CCl_4$); IR: $\nu_{OH}$ 3580 and 3540 cm$^{-1}$, 1615, 1495, 1445, 1385, 1363, 1350, 1330, 1298, 1275, 1240, 1203, 1190, 1170, 1130, 1098, 1012, 1004, 970, 952, and 865 cm$^{-1}$; UV: $\lambda_{max}$ 210 ($\epsilon$ = 18000), 278 ($\epsilon$ = 2736) and 310 ($\epsilon$ = 1415), shifted to 234 ($\epsilon$ = 4090), 246 ($\epsilon$ = 4340), 282 ($\epsilon$ = 1570) and 335 nm ($\epsilon$ = 1006) in alkaline MeOH; MS: M$^+$ 300.2089 ($C_{20}H_{28}O_2$, requires: 300.2089; 100), 285 (28), 257 (30), 244 (45), 231 (39), 229 (40), 215 (31), 204 (38), 201 (31), 191 (25), 189 (18), 179 (21) and 165 (43); $^1$H NMR: 0.85 (3H s 4$\beta$Me), 0.89 (3H s 4$\alpha$Me), 1.19 (6H d J = 7.0 Hz iPr<u>Me</u>), 2.82 (2H dd $W_{1/2}$ = 16 Hz C-7<u>H</u>$_2$), 3.09 (1H bd J = 15 Hz C-20<u>H</u>), 3.12 (1H hep J = 7.0 Hz iPrC<u>H</u>), 3.72 (1H d J = 15 Hz C-20H′), 5.50 (2H m disappear on $D_2O$ exchange, 2 × O<u>H</u>), 5.51 (1H bt J = 3.0 Hz C-1<u>H</u>) and 6.52 ppm (1H bs finer on $D_2O$ exchange, C-14<u>H</u>); $^{13}$C-NMR: 140.25 (s), 139.03 (s), 137.90 (s), 134.30 (s), 131.28 (s), 124.51 (s), 120.89 (d), 117.55 (d), 50.60 (d), 35.24 (t), 34.36 (t), 32.01 (s), 31.17 (t), 30.41 (t), 27.43 (q), 27.06 (d), 27.06 (q), 23.11 (t), 22.70 (q) and 22.53 (q).

*Acetylation of barbatusol*

Barbatusol (I, 150 mg) in pyridine (4 ml) was treated at room temp with $Ac_2O$ (4 ml). After 2½ hr, the reaction was diluted with water and extracted with $CHCl_3$. The organic layer was washed successively with 1H HCl, 5% $Na_2CO_3$ aq and brine. The organic layer was then dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by silica gel column chromatography (elution: gradient of AcOEt in hexane from 0 to 10%). Diacetate III was obtained pure (137 mg): III: non crystalline colorless gum; $[\alpha] = \frac{589 \quad 578 \quad 546\,nm}{-97.2 \quad -101.6 \quad -114.6^\circ}$ ($c$ = 2.01 in $CHCl_3$); IR: no OH absorption, $\nu_{C=O}$ 1760 cm$^{-1}$, 1430, 1375, 1300, 1240, 1190, 1030, 975 and 895 cm$^{-1}$; UV (cyclohexane): $\lambda_{max}$ 228 ($\epsilon$ = 3495), 268 ($\epsilon$ = 1685), 275 ($\epsilon$ = 1810), 288 ($\epsilon$ = 1620) and 296 nm ($\epsilon$ = 1390); MS: M$^+$ 384.2293 (10, $C_{24}H_{32}O_4$ requires: 384.2301), 342 (100), 300 (51), 282 (53), 257 (12), 244 (17), 231 (10), 229 (9), 215 (8), 204 (12), 201 (9), 191 (7) and 165 (13); $^1$H NMR: 0.88 and 0.90 (3H each s 4$\alpha$,4$\beta$Me), 1.19 and 1.20 (3H each d J = 7.0 Hz iPr<u>Me</u>), 2.29 and 2.30 (3H each s OAc), 2.87 (2H m $W_{1/2}$ = 9 Hz C-7<u>H</u>$_2$), 2.87 (1H hep J = 7.0 Hz iPrC<u>H</u>), 3.05 and 3.48 (1H each d J = 16 Hz C-20 <u>H</u>,H′), 5.41 (1H t J = 4 Hz C-1 <u>H</u>) and 6.93 ppm (1H s C-14 <u>H</u>); $^{13}$C NMR: 140.26 (s), 138.19 (s), 138.10 (s), 136.63 (s), 131.42 (s), 131.35 (s), 124.12 (d), 120.93 (d), 50.59 (d), 36.15 (t), 34.86 (t), 32.02 (s), 30.83 (t), 29.85 (t), 27.63 (d), 27.38 (q), 26.92 (q), 23.04 (t + q), 22.94 (q), acetate signals at 168.46 & 168.14 (each s) and 20.40 (q + q).

*Oxidation of barbatusol*

Barbatusol (I, 5 mg) in anhyd diethyl-ether (10 ml) was oxidized with $Ag_2O$ (10 mg) freshly prepared. The medium immediately turned from light yellow to deep red. Filtration of the medium followed by evaporation under reduced pressure yielded pure *o*-quinone (by TLC) IV was a reddish unstable gum:

*Compound* IV. IR: no OH absorption, 2940, $\nu_{C=O}$ 1670 and 1645 cm$^{-1}$, $\nu_{C=C}$ 1550 cm$^{-1}$, 1445, 1385, 1355, 1270 cm$^{-1}$; UV: $\lambda_{max}$ 227 ($\epsilon$ = 8380), 270 ($\epsilon$ = 4095) and 438 nm ($\epsilon$ = 1000); MS: M$^+$ 298.1918 (30, $C_{20}H_{26}O_2$ requires: 298.1933), 283 (9), 255 (9), 244 (13), 242 (10), 230 (12), 228 (12), 215 (10), 165 (24), 149 (27), 141 (11) and 43 (100).

*Catalytic hydrogenation of barbatusol*

Barbatusol (I, 22 mg) in AcOEt (5 ml) was hydrogenated at atmospheric pressure of $H_2$ on 10% Pd/C. After 5 hr, the medium was filtered over a 5-cm silicagel column (AcOEt as eluent). Evaporation of the filtrate under reduced pressure afforded VI as a mixture of epimers at C-1:

*Compound* VI. IR: $\nu_{OH}$ 3400; 2950, 1645 (w), 1460, 1395, 1370, 1320, 1300, 1265, 1250, 1080, 980 and 910 cm$^{-1}$; UV: $\lambda_{max}$ 234, 280 and shoulder at 307 nm; MS: M$^+$ 302 (21, $C_{20}H_{30}O_2$), 287 (16), 272 (10), 257 (9), 179 (21) and 178 (26); $^1$H NMR: complex Me region:

0.70 and 0.80 (both s 4βMe), 0.89 and 0.93 (both s 4αMe), 1.13 (6H d J = 7.0 Hz iPrMe), 2.93 (1H hep J = 7.0 Hz iPrCH) and 6.53 ppm (1H s C-14 H).

*Methylation of barbatusol*

Barbatusol (I, 200 mg), dissolved in acetone (10 ml), was treated with MeI (2 ml) and $K_2CO_3 \cdot 2H_2O$ (2 g) at room temp. during 1 day. The solids were then filtered off, washed with acetone and the filtrate evaporated to dryness. The residue was purified by silica gel column chromatography (eluent: gradient of AcOEt in hexane from 0 to 10%). Compound XII was obtained as light yellow solid (XII) (78 mg):

*Compound* XII, amorphous $|\alpha| = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{-85.0 \quad -88.4 \quad -99.7 \quad -159.7°}$ ($c$ = 1.00 in $CHCl_3$); IR: no OH absorption, 2940, 1475, 1445, 1404, 1328, 1300, 1222, 1125, 1095, 1078, 1045, 1000, 970, 940 and 875 $cm^{-1}$; UV: $\lambda_{max}$ 232 and 263 nm; MS: $M^+$ 328 (98, $C_{22}H_{32}O_2$), 313 (14), 300 (2), 297 (7), 285 (26), 272 (28), 259 (16), 257 (12), 229 (12), 193 (57) and 149 (16); $^1H$ NMR: 0.89 (3H s 4βMe), 0.93 (3H s 4αMe), 1.21 (6H d J = 7.0 Hz iPrMe), 2.82 (2H m $W_{1/2}$ = 17 Hz C-7H$_2$), 3.05 (1H bd J = 16 Hz C-20 H), 3.27 (1H hep J = 7.0 Hz iPrCH), 3.82 (1H d J = 16 Hz C-20 H'), 3.83 and 3.85 (3H each s C-11 and C-12 OMe), 5.49 (1H bt J = 3 Hz C-1 H) and 6.69 ppm (1H s C-14 H).

*Isolation of carnosol*

Dry leaves of *Rosmarinus officinalis* (152g), collected at Rio de Janeiro, were extracted exhaustively with $CH_2Cl_2$ in a Soxhlet apparatus. The crude extract (18g) was dissolved in MeOH; the MeOH insoluble part of the extract was filtered off. The filtrate was concentrated under reduced pressure and was repeatedly filtered on a 250-ml bed column of Sephadex LH-20, eluting with pure MeOH. Crude VII (1.49g) was obtained in that way. Two crystallizations from AcOH afforded pure VII identified by physico-chemical and spectroscopic data.[13,14]

*Methylation of carnosol*

Carnosol VII (252 mg) was treated in the same way as for I (see above). TLC examination of the mixture (eluent: hexane–AcOEt 3%) showed the presence of two dominant compounds. Separation by silica gel column chromatography (eluent: hexane–AcOEt from 100:0 to 90:10) afforded 56 mg of XIV (yield: 21%) and 150 mg of VIII (yield: 53%).

*Compound* VIII. Oil; $|\alpha| = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{-44.2 \quad -45.4 \quad -48.9 \quad -43.6°}$ ($c$ = 1.00 in $CHCl_3$); IR: no OH absorptions, 2940, $\nu_{C=O}$ 1735 $cm^{-1}$, $\nu_{C=O}$ 1220 $cm^{-1}$ and 1050 $cm^{-1}$, 950, 885 and 853 $cm^{-1}$; UV: $\lambda_{max}$ 240 ($\epsilon$ = 14000), 280 ($\epsilon$ = 8900) and 310 nm (shoulder); MS: $M^+$ 372.2287 (59, $C_{23}H_{32}O_4$ requires: 372.2301), 327 (8), 313 (100), 271 (90), 256 (30), 243 (77), 228 (26), 201 (44), 149 (19), 84 (64) and 55 (28); $^1H$ NMR: 0.89 (3H s 4βMe), 1.03 (3H s 4αMe), 1.19 and 1.22 (3H each d J = 7.0 Hz iPrMe), 2.56 (1H dd J = 2.8 and 3.0 Hz C-5αH), 3.25 (1H hep J = 7.0 Hz iPrCH), 3.55 (3H s COOMe), 3.76 and 3.80 (3H each s C-11 and C-12 OMe), 6.01 (1H dd J = 2.8 and 9.5 Hz C-6 H), 6.36 (1H dd J = 3.0 and 9.5 Hz C-7 H) and 6.67 ppm (1H s C-14 H).

*Compound* XIV. m.p. 156–158° (lit. 156° and 155–156°[14]); $|\alpha|_D$ = −75.6° ($c$ = 1.00 in $CHCl_3$) (lit. −73.5°[14]); IR: no OH absorption, 2945, $\nu_{C=O}$ 1740 $cm^{-1}$, $\nu_{C-O}$ 1270 $cm^{-1}$ and $\delta_{C-H}$ 850 $cm^{-1}$; UV: $\lambda_{max}$ 241 ($\epsilon$ = 6700) and 273 nm ($\epsilon$ = 750); MS: $M^+$ 358 (15, $C_{22}H_{30}O_4$), 314 (100), 299 (23), 284 (4), 271 (5), 245 (12), 243 (10), 232 (17), 229 (13), 215 (10), 201 (9) and 189 (12); $^1H$ NMR: 0.87 (3H s 4βMe), 0.92 (3H s 4αMe), 1.19 and 1.20 (3H each d J = 7.0 Hz iPrMe), 3.31 (1H hep J = 7.0 Hz iPrCH), 3.82 and 3.84 (3H each s C-11 and C-12 OMe), 5.43 (1H dd J = 4.0 and 1.5 Hz C-7 H) and 6.87 ppm (1H s C-14 H).

*Hydrogenation of* VIII

*Compound* VIII (136 mg), dissolved in AcOEt (15 ml), was treated with $H_2$ at a pressure of 45 psi and room temp. with 10% Pd/C as catalyst. After 3 hr, the medium was filtered over a 5-cm silica gel column eluted with pure AcOEt. IX was obtained in 96% yield and was pure by TLC:

*Compound* IX. Oil; $|\alpha| = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\,nm}{+135 \quad +139 \quad +159 \quad +286 \quad +486°}$ ($c$ = 1.00 in $CHCl_3$); IR: $\nu_{C=O}$ 1720 $cm^{-1}$, $\nu_{C-O}$ 1230 and 1040 $cm^{-1}$, 965, 940, 915, 860 and 845 $cm^{-1}$; UV: $\lambda_{max}$ 234 and 279 nm; MS: $M^+$ 374.2442 (47, $C_{23}H_{34}O_4$ requires 374.2457), 315 (100), 299 (6), 273 (12), 259 (10), 245 (35), 233 (31), 219 (87), 206 (31) and 69 (27); $^1H$ NMR: 0.79 (3H s 4βMe), 0.97 (3H s 4αMe), 1.20 (6H d J = 7.0 Hz iPrMe), 2.84 (2H dd J = 2.8 and 8.4 Hz C-7H$_2$), 3.22 (1H hep J = 7.0 Hz iPrCH), 3.65–3.66 and 3.76 (3H each s COOMe and C-11 + C-12 OMe) and 6.66 ppm (1H s C-14 H).

*LAH reduction of* IX

*Compound* IX (100 mg), dissolved in dry THF (5 ml), was refluxed during 3 hr in the presence of $H_4LiAl$ (155 mg). The medium was then allowed to cool and added successively AcOEt, EtOH, water and 2N $H_2SO_4$ (6 ml). Extraction with $CHCl_3$ and evaporation of the dried organic layer afforded a residue composed of at least three products (TLC). Purification by column chromatography on silica gel (eluent: gradient of AcOEt in hexane from 5 to 50%) furnished pure X (58 mg, yield: 60%) and pure XVI (22 mg, yield: 23%):

*Compound* X. m.p. 82–84° (lit. 85–86°[12]); UV: $\lambda_{max}$ 235 and 279 nm; $|\alpha| = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\,nm}{+108.7 \quad +113.6 \quad +130.0 \quad +231.7 \quad +388.7°}$ ($c$ = 1.00 in $CHCl_3$); IR: $\nu_{OH}$ 3400 $cm^{-1}$, no CO absorption, $\nu_{C-O}$ 1250 and 1050 $cm^{-1}$, 970, 930, 890, 880 and 870 $cm^{-1}$; MS: $M^+$ 346 (12, $C_{22}H_{34}O_3$), 315 (100), 285 (9), 273 (10), 259 (8), 245 (32), 233 (28), 219 (72), 165 (14), 136 (13) and 69 (23); $^1H$ NMR: 0.97 (6H s 4α, 4βMe), 1.17 and 1.21 (3H each d J = 7.0 Hz iPrMe), 2.87 (2H dd J = 7.8 and 3.0 Hz C-7 H$_2$), 3.24 (1H hep J = 7.0 Hz iPrCH), 3.74 (1H d J = 11 Hz C-20 H), 3.74 and 3.88 (3H each s C-11 and C-12 OMe), 4.13 (1H bd J = 11 Hz C-20 H', sharpens on $D_2O$ addition) and 6.70 (1H s C-14 H).

*Compound* XVI. m.p. 208–210° (lit. 210–211°[12]); UV: $\lambda_{max}$ 232 and 278 nm; $|\alpha| = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{+92.3 \quad +96.8 \quad +111.5 \quad +200.7°}$ ($c$ = 1.00 in $CHCl_3$); IR: $\nu_{OH}$ 3000 (broad), $\nu_{C=O}$ 1705, $\nu_{C-O}$ 1255 and 1040, 965, 945 and 870 $cm^{-1}$; MS: $M^+$ 360 (25, $C_{22}H_{32}O_4$), 332 (8), 315 (28), 301 (51), 272 (10), 259 (13), 245 (35), 233 (22), 231 (32), 219 (84), 206 (32) and 205 (48); $^1H$ NMR: 0.84 (3H s 4βMe), 0.97 (3H s 4αMe), 1.19 and 1.21 (3H each d J = 7.0 Hz iPrMe), 2.82 (2H dd J = 10 and 4.0 Hz C-7 H$_2$), 3.22 (1H hep J = 7.0 Hz iPrCH), 3.67 and 3.78 (3H each s C-11 and C-12 OMe) and 6.65 ppm (1H s C-14 H).

*Treatment of* X *with* TsCl

*Compound* X (45 mg), in dry pyridine (5 ml), was treated at room temp. during 20 hr with an excess of TsCl. The medium was then diluted with water and extracted with $CHCl_3$. The dried organic layer was evaporated under reduced pressure. Purification of the crude reacted mixture by silica gel column chromatography (eluent: gradient of AcOEt in hexane from 0 to 5%) yielded 42 mg of XI, homogenous in TLC in various solvent systems and with the same $R_f$ as XII.

*Compound* $XI_{a+b}$, oil; GLC (3% OV-17): major peak at 6 min 35" (87%) accompanied by an impurity (13%) of RT 7 min 32" (Flow of carrier gas: 45 ml/min); UV: $\lambda_{max}$ 232, 264 and 298 nm; $|\alpha| = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\,nm}{-70 \quad -74 \quad -84 \quad -153 \quad -251°}$ ($c$ = 1.00 in $CHCl_3$); IR: no OH absorption, no CO absorption, $\nu_{C-O}$ 1220 and 1050 $cm^{-1}$, 1000, 970, 940 and 860 $cm^{-1}$; MS: $M^+$ 328 (100, $C_{22}H_{32}O_2$), 313 (14), 297 (4), 285 (13), 272 (15), 259 (19), 257 (9), 229 (9), 193 (22), 177 (5), 141 (4), 105 (4), 95 (8), 91 (4) and 69 (5); $^1H$ NMR: 0.87 (2H s 4βMe of $XI_a$), 0.91 (2H s 4αMe of $XI_a$), 0.99 (2H s 4α, 4βMe of $XI_b$), 1.20 (6H d J = 7.0 Hz iPrMe), 2.85 (2H dd $W_{1/2}$ = 17 Hz C-7 H$_2$), 3.02 (1H bd J = 15 Hz C-20 H), 3.25 (1H hep J = 7.0 Hz iPrCH), 3.81 (1H d J = 15 Hz), 3.83 (6H s C-11 and C-12 OME), 5.47 (0.6 H bt J = 3.0 Hz C-1 H of $XI_a$) and 6.68 ppm (1H s C-14 H).

*Demethylation of* $XI_{a+b}$

*Compound* $XI_{a+b}$ (24 mg), dissolved in dry $CH_2Cl_2$ (5 ml), was treated at room temp. during 10 min with 10 drops of freshly distilled $BBr_3$. The medium was then diluted by careful addition of distilled water and extracted with $CHCl_3$. The dried organic layer was evaporated under reduced pressure and the

crude residue was purified by preparative silica gel TLC (eluent: hexane AcOEt 7%). This yielded 8 mg of the mixture of double bond isomers XIII identical in TLC with natural I.

*Compound* XIII. Gum; $|\alpha| = \frac{589 \quad 578 \quad 546\,nm}{-12.0 \quad -17.4 \quad -45.3^\circ}$ ($c = 0.33$ in $CHCl_3$); UV: $\lambda_{max}$ 222, 278 and 312 nm; IR: $\nu_{OH}$ 3448 $cm^{-1}$, 2924, 2825, 1628, 1493, 1440, 1380, 1362, 1325, 1275, 1215, 1160, 1110, 1085, 1000, 970 (weak), 935 and 870 $cm^{-1}$; MS; highest *m/z* 300 (98, $C_{20}H_{28}O_2$), 285 (28), 271 (5), 257 (23), 244 (14), 231 (100), 229 (26), 215 (30), 204 (17), 191 (20), 189 (18), 179 (34), 178 (34), 165 (19) and 149 (15); $^1H$ NMR: 0.72 (s), 0.87 (s), 0.94 (s), 0.98 (s), 1.23 and 1.25 (each d J = 7.0 Hz), 3.12 (m), 3.60 (bs exchangeable by $D_2O$), 5.50 (bt) and 6.43 ppm (complex).

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## REFERENCES

[1]R. Zelnik, D. Lavie, E. C. Levy, A. H. J. Wang and I. C. Paul, *Tetrahedron* **33**, 1457 (1977).
[2]P. Rüedi and C. H. Eugster, *Helv. Chim. Acta* **55**, 1994 (1972); and **56**, 1129 (1973).
[3a]J. S. Tandon *et al. Ind. J. Chem.* **15b**, 880 (1977); **16b**, 341 (1978); **16B**, 1055 (1978); **17B**, 321 (1979) and **18B**, 214 (1979); [b]S. V. Bhat, B. S. Bajwa H. Dornauer, N. J. De Souza and H. Fehlhaber, *Tetrahedron Letters* 1669 (1977).
[4]M. P. Dubey, R. C. Srinal, S. Nityanand and B. N. Dhawan, *J. Ethnopharmacol.* **3**, 1 (1981).
[5]K. B. Seamon, W. Padgett and J. W. Daly, *Proc. Natl Acad. Sci. U.S.A.* **78**, 3363 (1981).
[6]A. Kelecom and I. C. Sobreiro-Kelecom, *Ciência e Cult.* (Supl), *VII Cong. Bras. Med. Plants*, Belo Horizonte (1982), in press
[7]In ref 6, I was named "barbatol"; this name is however not available.
[8]A. I. Scott, *Interpretation of Ultraviolet spectra of Natural Products*, pp 91–97. Pergamon, Oxford (1964).
[9]T. Miyase, P. Rüedi and C. H. Eugster, *Helv. Chim. Acta* **60**, 2789 (1977).
[10]M. Yatagai and T. Takahashi, *Phytochem.* **18**, 176 (1979).
[11]T. Matsumoto, S. Usui and T. Morimoto, *Bull. Chem. Soc. Jap.* **50**, 1575 (1977).
[12]C. H. Brieskorn, A. Fuchs, J. B.-son Bredenberg, J. D. McChesney and E. Wenkert, *J. Org. Chem.* **29**, 2293 (1964).
[13]C. H. Brieskorn and A. Fuchs, *Chem. Ber.* **95**, 3034 (1962).
[14]H. Linde, *Helv. Chim. Acta* **47**, 1234 (1964).
[15]W. L. Meyer, R. A. Manning, E. Schindler, R. S. Schroeder, and D. C. Shew, *J. Org. Chem.* **41**, 1005 (1976).
[16]L. F. Fieser and M. Fieser *Reagents for Organic Synthesis*, Vol. I, p 585. Wiley, New York (1967).
[17]T. Kametani, H. Matsumoto, T. Honda and K. Fukumoto, *Tetrahedron Letters* 2379 (1981).
[18]M. Yatagai and T. Takahashi, *Phytochem.* **19**, 1149 (1979).
[19]J. F. W. McOmie, M. L. Watts and D. E. West, *Tetrahedron* **24**, 2289 (1968).
[20]X. A. Dominguez, H. Gonzalez, R. Argon, M. Gutiérrez, J. S. Marroquin and W. Watson, *Planta Med.* **30**, 237 (1976).
[21a]C. W. Brandt and L. G. Neubauer, *J. Chem. Soc.* 1031 (1939). [b]C. R. Enzell and I. Wahlberg, *Acta Chem. Scand.* **23**, 871 (1969); [c]T. Nishida, I. Wahlberg and C. R. Enzell, *Org. Magn. Res.* **9**, 203 (1977).