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MINOR COMPONENTS WITH THE γ-CYCLOGERANIL GERANIOL SKELETON FROM *BELLARDIA TRIXAGO* (L.) ALL.

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Abstract—Trixagol 1 and fourteen derivatives with the skeleton of γ-cyclogeranil geraniol were isolated from *Bellardia trixago*, the structures were determined by spectroscopic methods and confirmed by partial synthesis. Furthermore, 3,4-dihydro-γ-ionone, α-ambrinol, β-sitosterol and three flavonoids 5-*O*-demethyl tangeretin, 5-hydroxy auranetin and 3'-methoxy calycopterin were also isolated.

Bellardia trixago (L.) All (= *Trixago apula*, *Bartsia trixago*, *B. versicolor*) Fam. *Scrophulariaceae*, subfam. *Rhinanthoidea* is a common plant in the Mediterranean region which has two varieties, one with white and purple flowers (var. *versicolor* (Willd) P. Coutinho) and the other with yellow flowers (var. *flaviflora* (Boiss) Maire).

A number of Iridoids, characteristic substances in *Scrophulariaceae*, have been found in *B. trixago*: aucuboside,[1] aucubine[2] and bartsioside[3] have already been identified in this plant but, as far as we know, no details of the remaining components have yet been published.

We reported in a previous communication[4] the structural identification of a diterpene alcohol **1** for which the name of trixagol was proposed. This substance with a retinane skeleton is the main component of the hexane extract of *B. trixago* var. *versicolor* and var. *flaviflora*.

We now describe the minor components of the hexane extract from *B. trixago* var. *versicolor* and var. *flaviflora* whose structures have been established by spectroscopic methods and confirmed by partial synthesis.†

The hexane extract of *B. trixago* var. *versicolor* was defatted with methanol and methanol saturated with urea and the extracted with 10% NaOH. The alkaline fraction (12%) on acidification with CO_2 gives a yellow precipitate identified as 3'-methoxy calycopterin.[5] The neutral fraction (38.4%) was chromatographed on SiO_2 and, after purification, 17 substances were identified. These were 3,4-dihydro-γ-ionone,[6] α-ambrinol,[6] β-sitosterol,[7] 5-*O*-demethyl tangeretin,[8] 5-hydroxy auranetin[9] and 12 new natural substances (**1 2a 2b 3 4 5 6 7a 8a 9a 10a 11a**).

The hexane extract from *B. trixago* var. *flaviflora* was elaborated as the extract from var. *versicolor*. The fat content is higher in var. *flaviflora* (53% vs 47%) and also esters **2c 2d** and **2e** were isolated instead of **2b**. The remaining components were the same as in var. *versicolor*.

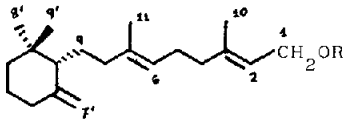
The main component of the neutral fraction from the hexane extract in both plants (49%) is, as we said above, the alcohol named trixagol **1** whose structure and stereochemistry were established as (2E, 6E, 1'S) 3,7-dimethyl-9(6,6-dimethyl-2-methylencyclohexyl) nona-2,6-dien-1-ol. All the other components herein described, β-sitosterol and flavonoids excluded, are structurally related to trixagol.

Trixagoyl acetate **2a** and trixagoene **3** were isolated as natural substances from the less polar fractions of the chromatography and were also described in our first report. Acetylation of **1** gives the acetate identical with natural **2a**. Otherwise; trixagoene **3** was synthesized through dehydration of trixagol **1** by alkaline fusion of trixagol with KOH.[10,11] Apart from trixagoene the last reaction gives the alcohols **12a** and **13a** arising from the oxidation-hydrogenation of trixagol.[12]

Compound **4**, Isotrixagol, showed IR absorptions characteristic of a tertiary allylic hydroxyl (3400, 1105 cm^{-1}) as well as a vinyl radical (3010, 1660, 990, 920 cm^{-1}) also evident in the ^1H NMR spectrum (see Table 1). The similarity of the spectra with those of **1**, the absence of a primary alcohol, as well as MS data, led us to assign an γ-cyclogeranyl linalool structure for alcohol **4**. This structure was confirmed by the isomerization of **1** into **4**, following the Wharton procedure.[13] Oxidation of **1** with PDC[14] yields the aldehyde **15** which was treated with H_2O_2 at pH = 8.[15] The product α,β-epoxyaldehyde **16**, was treated with hydrazine hydrate in glacial acetic acid at room temperature giving the expected isotrixagol **4**, which had identical spectra to those of natural isotrixagol, and compound **14** as main products. This synthetic substance showed IR absorptions characteristic of a tertiary allylic hydroxyl (3360, 1120 cm^{-1}) and ^1H NMR signals (Table 1) at 1.21 (s, 3H, CH_3–C–OH), 2.55 (m, 1H, =C–CH), 5.63 and 5.67 (2br s 2H, $^{H}\!>\!C\!=\!C\!<^{H}$). From these data we conclude that the structure of this second isomerization product is that shown in **14**. Similar cyclization products have been found as subproducts of other Wharton isomerizations **16**. The configuration of C_3 in **4** is R by variation in molecular rotation of **4** to **1** $\Delta M_D = +11.7°$ **17**.

Isotrixagoyl oxide **5** had the molecular formula $C_{20}H_{32}O$ indicating the presence of five unsaturation equivalents. The IR spectrum showed absorptions characteristic of a terminal methylene and a radical vinyl (3060, 1640, 985, 920, 890 cm^{-1}). The ^1H NMR spectrum of **5** was similar to that of isotrixagol, **4**, with the exception of the presence of a new allylic α-O proton signal at 4.20 (m, 1H) and another signal at 4.72 and 4.95 (s, 2H, $>C=CH_2$). The structure of **5** was confirmed by synthesis. Epoxidation of **2** with *m*-chloro perbenzoic acid gives **7b** which on cyclization with perchloric acid[18]

†This work was presented *XIIth IUPAC International Symposium on Natural Products Chemistry*, 21–27 September 1980. Puerto de la Cruz, Tenerife, Canary Islands, Spain.



1 R= H

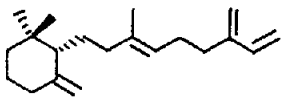
2a R= Ac

2b R= $COCH_2COOEt$

2c R= $COCH_2COOMe$

2d R= $COCH_2COOi\text{-}Bu$

2e R= $COCH_2COOTrixagoyl$



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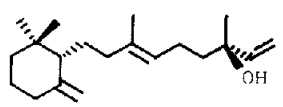

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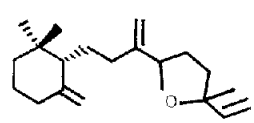
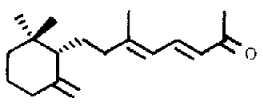


5



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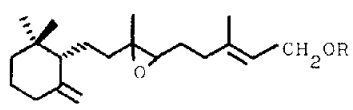
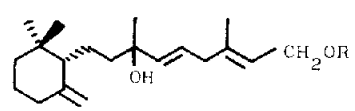


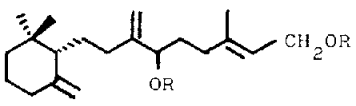
7a R= H

7b R= Ac



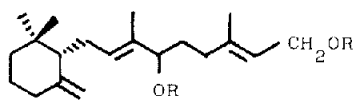
8a R= H

8b R= Ac



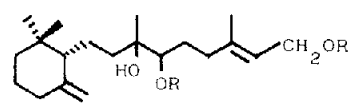
9a R= H

9b R= Ac



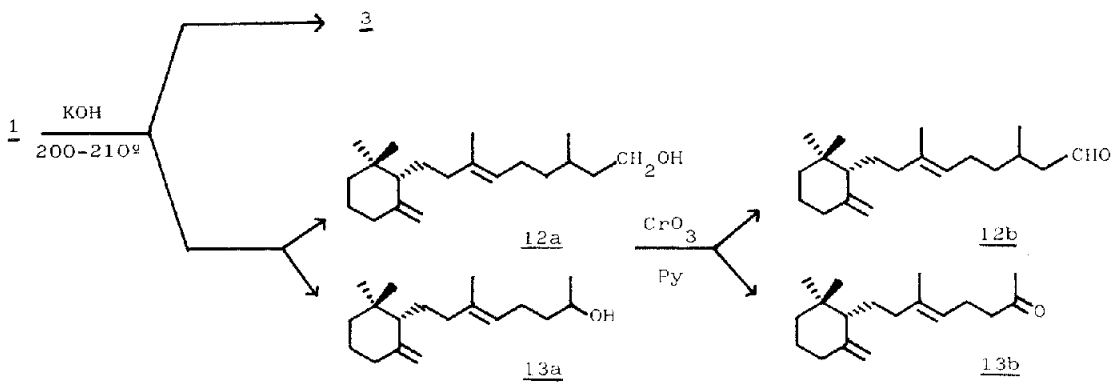
10a R= H

10b R= Ac



11a R= H

11b R= Ac



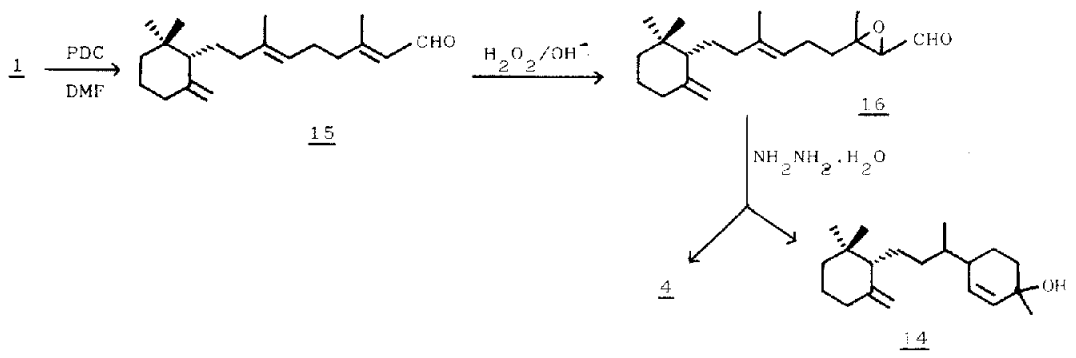


Table 1. ^{1}H NMR chemical shifts (δ values from TMS) and multiplicities (J values in Hz) of trixagol derivatives (in CCl_4) signals

Cpd	1-H	2-H	4-H	5-H	6-H	8-H	10-H	11-H	7'-H	8'-H,9'-H	Other signals $-OCOCH_2COO-$	
2a	4.45 (d,7)	5.28 (t,7)			5.02 (t,7)		1.70 (s)	1.58 (s)	4.49,4.71 (s)	0.82,0.91 (s)		-OAc 1.93(s)
2b	4.51 (d,7)	5.27 (t,7)			4.98 (t,7)		1.69 (s)	1.57 (s)	4.48,4.67 (s)	0.83,0.90 (s)	3.19 (s)	$-COOCH_2CH_3$ 1.25(t,6) 4.12(c,6)
2c	4.51 (d,7)	5.28 (t,7)			5.02 (t,7)		1.70 (s)	1.55 (s)	4.50,4.65 (s)	0.85,0.95 (s)	3.23 (s)	$-COOCH_3$ 3.65(s)
2d	4.51 (d,7)	5.28 (t,7)			5.01 (t,7)		1.71 (s)	1.57 (s)	4.46,4.66 (s)	0.83,0.90 (s)	3.20 (s)	$-COOCH_2CH(CH_3)_2$ 0.93(d,7) 3.83(d,7)
2e	4.59 (d,7)	5.30 (t,7)			5.01 (t,7)		1.75 (s)	1.60 (s)	4.51,4.70 (s)	0.85,0.95 (s)	3.25 (s)	
3	5.00-5.40 (m)	6.30 (dd,11,17)			5.00-5.40 (m)		4.91 (s)	1.52 (s)	4.49,4.69 (s)	0.81,0.89 (s)		
4	4.80-5.30 (m)	5.83 (dd,11,17)			5.00 (m)		1.20 (s)	1.56 (s)	4.49,4.69 (s)	0.82,0.90 (s)		
5	4.70-5.30 (m)	5.82 (dd,11,17)			4.20 (m)		1.28 (s)	4.72,4.95 (s)	4.50,4.70 (s)	0.87,0.95 (s)		
7a	4.03 (d,7)	5.30 (t,7)			2.60 (t,6)		1.65 (s)	1.20 (s)	4.50,4.69 (s)	0.85,0.95 (s)		
8a	3.99 (d,7)	5.32 (t,7)	2.65 (m)	5.44 (s,$w_{1/2}$=6)	5.44 (s,$w_{1/2}$=6)		1.61 (s)	1.20 (s)	4.49,4.66 (s)	0.82,0.90 (s)		
9a	3.99 (d,7)	5.31 (t,7)			3.90 (m)		1.61 (s)	4.72,4.93 (s)	4.50,4.71 (s)	0.85,0.91 (s)		
10a	4.03 (d,6)	5.39 (t,6)			3.86 (m)	5.28 (t,6)	1.61 (s)	1.65 (s)	4.53,4.74 (s)	0.84,0.95 (s)		
11a	4.05 (d,7)	5.40 (t,6)			3.90 (m)		1.67 (s)	1.09 (s)	4.52,4.71 (s)	0.89,0.95 (s)		

produces **17** which can be dehydrated with phosphorus oxychloride to give Isotrixagoyl oxide **5** identical in all respects to the natural product.

Four malonates of trixagoyl and other alcohols **2b**, **2c** **2d** and **2e** were isolated succesively. The presence of a malonate in these molecules is established by its IR absorptions at 1750 and 1730 cm^{-1} and ^1H NMR signals at 3.20 (s, 2H–OCO–CH_2–COO–). Other signals which determine the structure of a second alcohol which esterifies the malonic acid are: In **2b** 4.12 (qu, 2H, J = 6Hz) and 1.25 (t, 3H, J = 6 Hz) of an ethyl group; **2c** 3.65 (s, 3H) of the methyl group; **2d** 3.83 (d, 2H, J = 7 Hz), 0.93 (d, 6H, J = 7 Hz) of an isobutyl radical; **2e** signals of ditrixagoyl radical (Table 1).

Dinortrixagone **6** had the molecular formula $C_{18}H_{28}O$ and its structure has been established by the following spectroscopic properties. In the IR spectrum, it shows absorptions for a $\alpha\beta$, $\gamma\delta$ unsaturated carbonyl at 1660 cm^{-1} and double bonds at 3030, 3010, 1625, 1585, 970 cm^{-1}. The ultraviolet band at 291 nm was consistent with a conjugated dienone with two substituents in δ.[19] The ^1H NMR spectrum shows five signals due to the –C(Me)=CH–CH=CH–CO–CH_3 group at δ 1.85 (s, 3H) 5.85 (d, 1H, J = 11 Hz), 5.91 (d, 1H, J = 15 Hz), 7.25 (qu, 1H, J_1 = 11 Hz J_2 = 15 Hz). The coupling constants J_1 = 11 Hz and J_2 = 15 Hz for olefinic protons are indicative of a "*trans*" stereochemistry for Δ^3.[20] The remaining signals (Table 1) are similar to those of the spectrum of **1**.

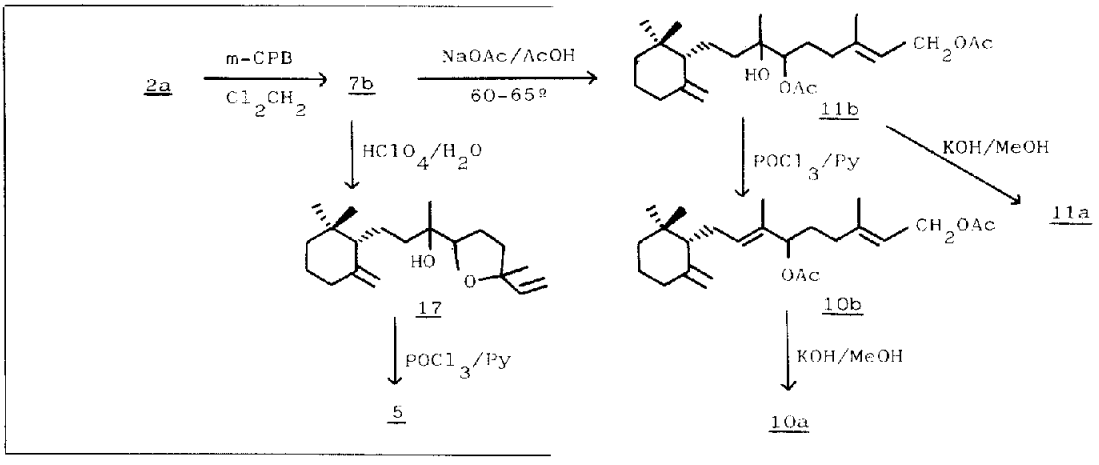
In 6,7-epoxy trixagol **7a**, the ^1H NMR signals at 1.20 (s, 3H) and 2.60 (t, 1H, J = 6 Hz) suggested that the relationship of **1** to **7a** was that of an epoxide to the corresponding olefin in Δ^6. The structure was confirmed by epoxidation of trixagoyl acetate with *m*-chloro perbenzoic acid, the product obtained **7b** was identical to the product of acetylation of **7a**.

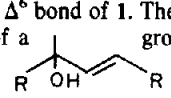
Trixagodiol B **9a**, has a hydroxyl allylic to a terminal disubstituted double bond, characterised by ^1H NMR signals at 3.90 (m 1H) due to the geminal –OH secondary protons and at 4.72 and 4.93 (2s, 2H) due to the terminal methylene $\Delta^{7,11}$ **9a** is readily acetylated to give the diacetate **9b** whose spectral data are in agreement with the proposed structure.

Diols **8a** and **9a** were synthesized by singlet oxygen oxidation of trixagol **1** and the synthetic substances have identical properties to those of the natural products except for their optical rotations $[\alpha]_D = 7.9^o$ $[\alpha]_D = 3.9^o$ respectively.

Trixagodiol C **10a** exhibits spectroscopic properties characteristic of a secondary –OH group allylic to a trisubstituted double bond: ^1H NMR signals at 3.86 (m, 1H) of a geminal OH proton, 1.65 (s, 3H) of the methyl C_{11} and 5.28 (t, 1H, J = 6 Hz) of the proton olefinic in C_8. On acetylation **8a** afforded the diacetate **8b**. The structure of trixagodiol C was confirmed by acetolysis of **7b** with acetic anhydride and sodium acetate, by dehydration and saponification of the resulting product it gives **10a**.

The most polar fraction is a complex mixture of polyols and after their acetylation we have isolated **11b** whose molecular formula is $C_{24}H_{40}O_5$. Its IR spectrum presents a non acetylable tertiary hydroxyl (3460, 1120 cm^{-1}) and acetate groups (1730, 1230, 1020 cm^{-1}). On saponification this gives trixagotriol **11a** with signals in the ^1H NMR at 3.90 (m, 1H) and 1.09 (s, 3H) characteristic of a glycol in the positions C_6–C_7. The existence of this group was demonstrated by treatment of **11a** with 2,2-dimethoxy propane and *p*-toluene sulphonic acid. The product resulting from the reaction is **17**, formed by protonation of the primary hydroxyl and gives, after dehydration, a tertiary allylic cation which on cyclisation affords **17**.



The isomeric diols **8a**, **9a**, **10a** had the molecular formula $C_{20}H_{34}O_2$ and correspond to oxidation products of the double Δ^6 bond of **1**. The trixagodiol A **8a** shows the presence of a  group, characterised by IR absorptions at 3360 and 1100 cm^{-1} (–OH) and 3010, 1660 970 cm^{-1} ($_H$>C=C<^{H}) and ^1H NMR signals at 1.20 (s, 3H, Me–C–OH), 5.44 (s $W_{1/2}$ = 6 Hz 2H) another at 2.65 (m, 2H) of the methylene C_4. Trixagodiol A on acetylation gives the monoacetate **8b** which after dehydration afforded the conjugated diene **18**, confirming the proposed structure **8a**.

EXPERIMENTAL

IR spectra were recorded on a Beckman IR-33 spectrophotometer. UV spectra were recorded in EtOH on a Beckman DK-2 spectrophotometer. ^1H NMR spectra were recorded on a Hitachi Perkin–Elmer R-24 (60 MHz) spectrometer using TMS as an internal standard in CCl_4. MS were obtained on a Hewlett-Packard Mod. 5930 A.

Collection and extraction of Bellardia trixago. 5 Kg. leaves and capsules of *B. trixago var. versicolar*, collected around Sancti-Spiritus, Salamanca (Spain) June 1978 were extracted with refluxing hexane for 24 h. giving 156 g. Dewaxing with MeOH and MeOH saturated with urea gave waxes (53 and 19.5 g. respectively) and the remaining product in Et_2O soln. was extracted with aq. 10% NaOH, to yield a neutral fraction 60 g.

The alkaline fraction, after acidification with CO_2, gives a precipitate of 3'-methoxy calycopterin 220 mg.

The neutral fraction was chromatographed on a dry column (Eluy. hexane–ether 1:1), separating seven fractions. Each of them was rechromatographed on silica gel or silicagel coated with $AgNO_3$ (20%) columns, to afford the different components, which were finally purified by preparative tlc or crystallization.

Fraction 1 (8.2 g) afforded **2a** (1.3 g), **2b** (76 mg), **3** (52 mg), **4** (210 mg), **5** (72 mg), **3,4-dihydro-γ-ionone** (110 mg).

Fraction 2 (2 g) afforded **6** (74 mg), **α-ambrinol** (82 mg).

Fraction 3 (27.5 g) is **1**.

Fraction 4 (1.5 g) afforded **β-sitosterol** (230 mg), **5-*O*-demethyl tangeretin** (430 mg).

Fraction 5 (2.7 g) afforded **7a** (75 mg).

Fraction 6 (2.8 g) afforded **5-hydroxy auranetin** (180 mg) **8a** (390 mg), **9a** (330 mg).

Fraction 7 (7.5 g) afforded **10a** (470 g) **11a** (2.3 g).

The collection of *B. trixago var. flaviflora* was carried out in Torrejon de Ardoz, Madrid (Spain) June 1979. 4.5 Kg leaves and capsules were extracted with refluxing hexane for 24 h. The procedure of extraction and isolation is analogous to the *var. versicolor*, with the following results: waxes (52.9%), neutral fraction (36.4%) and acid fraction (10.6%).

The following compounds are isolated from the neutral fraction: **1** (26 g), **2a** (1.5 g), **3** (75 mg), **4** (250 mg), **5** (30 mg), **6** (40 mg), **7a** (75 mg), **8a** (350 mg), **9a** (320 mg), **10a** (410 mg), **11a** (2.1 g), **3,4-dihydro-γ-ionone** (100 mg), **α-ambrinol** (125 mg), **β-sitosterol** (260 mg), **5-*O*-demethyl tangeretin** (3.4 g), **5-hydroxy auranetin** (150 mg) and also **2c** (143 mg), **2d** (1.7 g) and **2e** (32 mg).

Trixagoyl acetate **2a** eluted with hexane–ether (95:5) $[\alpha]_D$ +3.8° (c, 1.12, $CHCl_3$). IR (ν cm^{-1}) 3040, 1740, 1660, 1640, 1380, 1360, 1230, 1020, 950, 895, 850.

Trixagoene **3** was eluted with hexane. UV (EtOH) 225 nm IR (ν cm^{-1}) 3060, 1640, 1595, 975, 910, 890, 850.

Synthesis of **3** *from* **1**. **1** (2 g) was heated with finely powdered KOH (20 g) at 200–210° for 30 min. The resulting product was chromatographed on silica gel to afford **3** (420 mg), identical in all properties to the natural material, and a crude mixture of alcohols **12a** and **13a** (720 mg). Oxidation of **12a** and **13a**: CrO_3 (880 mg) in pyridine (18 ml) to a soln. of **12a**+**13a** in pyridine (6 ml) was added and the soln was stirred at room temperature for 4 h. The excess oxidant was destroyed with *i*-PrOH (1–2 drops) extracted with ether and washed with 2*N* HCl. The combined ether extracts were dried over Na_2SO_4 and the solvent evaporated to obtain, after chromatography on silica gel, **12b** (250 mg) and **13b** (310 mg). **12b** IR (ν cm^{-1}) 3040, 3010, 2720, 1730, 1660, 1650, 890, 850. ^{1}H NMR (δ ppm) 0.85 (s, 3H), 0.93 (s, 3H), 1.58 (s, 3H), 4.50 and 4.70 (2s, 2H), 4.98 (t, 1H, J = 6 Hz), 9.60 (t, 1H, J = 2 Hz). **13b** IR (ν cm^{-1}) 3070, 1725, 1660, 1640, 890, 840. ^{1}H NMR (δ ppm) 0.85 (s, 3H), 0.92 (s, 3H), 1.60 (s, 3H), 4.48 and 4.59 (2s, 2H), 4.95 (t, 1H, J = 6 Hz).

Isotrixagol **4** was eluted with hexane–ether (9:1) $[\alpha]_D$ +11.7° (c, 0.92, $CHCl_3$) IR (ν cm^{-1}) 3400, 3050, 3010, 1660, 1645, 1105, 990, 920, 890. Ms *m/e* (rel. int.) 272(14), 267(54), 199(41), 197(91), 175(100), 161(41), 149(43), 121(60), 109(58), 81(44).

Synthesis of **4**. To a solution of **1** (2 g) in DMF (10 ml) at −10° with strong agitation, PDC (4.5 g) was added and the soln was stirred continuously for 5 h at −10°. The product was poured into water and extracted with ether. The ethereal extracts were washed with 2*N* HCl and 5% $NaHCO_3$ aq. Removal of solvent afforded the residue (1.59 g), which was chromatographed on silica gel to afford **15** (894 mg) and **1** (612 mg). **15**: $[\alpha]_D$ +9.91° (c, 1.13, $CHCl_3$) IR (ν cm^{-1}) 3050, 3010, 2740, 1680, 1640, 1610, 1105, 890, 850. ^{1}H NMR (δ ppm) 0.84 (s, 3H), 0.92 (s, 3H), 1.60 (s, 3H), 2.17 (s, 3H), 4.51 and 4.73 (2s, 2H), 5.06 (m, 1H), 5.78 (d, 1H, J = 7 Hz), 9.91 (d, 1H, J = 7 Hz).

Epoxidation of **15**. To a soln of **15** (894 mg) in MeOH (10 ml) 30% H_2O_2 (1.15 ml) (pH ≃ 8 with 6*N* NaOH) was added. The soln was kept for 1½ h at 0°. When the MeOH had been evaporated H_2O was added and the organic phase extracted with ether. The residue (970 mg) was purified by chromatographie to give **16** (360 mg). **16**: IR (ν cm^{-1}) 3080, 3030, 2720, 1730, 1650, 1160, 895, 805. ^{1}H NMR (δ ppm) 0.86 (s, 3H), 0.92 (s, 3H), 1.43 (s, 3H), 1.60 (s, 3H), 3.02 (d, 1H, J = 6 Hz), 4.52 and 4.72 (2s, 2H), 5.04 (t, 1H, J = 6 Hz), 9.36 (d, 1H, J = 6 Hz).

Reduction of **16** *with hydrazine*. 98% $NH_2NH_2 \cdot H_2O$ (0.2 ml) in MeOH (1.5 ml) and glacial AcOH (0.1 ml) in MeOH (1.5 ml) to a soln of **16** (170 mg) in MeOH (3 ml) was added at room temperature. The colourless solution turned yellow; by tlc it is possible to verify that the reaction is instantaneous. The product (196 mg) by plc (hexane–ether 7:3) gave **4** (11.5 mg), identical to the natural one and **14** (24.5 mg). **14**: $[\alpha]_D$ +7.0° (c, 0.84, $CHCl_3$), IR (ν cm^{-1}) 3360, 3070, 3030, 3010, 1650, 1120, 890, 790, 765, 725. ^{1}H NMR (δ ppm) 0.86 (s, 3H), 0.93 (s, 3H), 1.01 (d, 3H, J = 6 Hz), 1.21 (s, 3H), 2.55 (m, 1H), 4.58 and 4.76 (2s, 2H), 5.63 and 5.67 (2*br* s, 2H).

Isotrixagoyl oxide **5** was eluted with hexane–ether (95:5), IR (ν cm^{-1}) 3060, 1640, 1160, 1030, 985, 920, 890. MS *m/e* (rel. int.) 288 (17.5), 273 (13.5), 255 (19.5) 220 (11), 205 (23), 149 (55), 109 (58), 81 (100).

Synthesis of **5**. To a stirred soln of **2a** (1 g) in CH_2Cl_2 (10 ml) at 0° *m*-CPB (560 mg) in CH_2Cl_2 (10 ml) was added and the mixture stirred for 4 h at 25°. Then a 10% soln of Na_2SO_3 was added dropwise and the organic phase was washed three times with 5% $NaCO_3$ aq. dried over Na_2SO_4 and evaporated to obtain **7b** (980 mg). IR (ν cm^{-1}) 3060, 3010, 1750, 1680, 1650, 1240, 1130, 1080, 1030, 960, 895, 850. ^{1}H NMR (δ ppm) 0.86 (s, 3H), 0.92 (s, 3H), 1.19 (s, 3H), 1.74 (s, 3H), 1.99 (s, 3H), 2.60 (t, 1H, J = 6 Hz), 4.50 (d, 2H, J = 6 Hz), 4.56 and 4.77 (2s, 2H), 5.28 (t, 1H, J = 6 Hz). **7b** (400 mg) in diglyme (2 ml) was treated with 2 drops of 60% $HClO_4$ and water and left to stand at room temperature for 19 h. The soln was extracted with $CHCl_3$ and the combined extracts washed three times with a 5% $NaHCO_3$ aq. dried over Na_2SO_4 and the solvent evaporated to obtain **17** (360 mg): IR (ν cm^{-1}) 3450, 3070, 1640, 1120, 990, 920, 890, 850. ^{1}H NMR (δ ppm) 0.88 (s, 3H), 0.92 (s, 3H), 1.12 (s, 3H), 1.26 (s, 3H), 3.70 (m, 1H) 4.48 and 4.69 (2s, 2H), 4.89 (dd, 1H, J_1 = 11 Hz J_2 = 2 Hz), 5.08 (dd, 1H, J_1 = 17 Hz J_2 = 2 Hz), 5.82 (c, 1H, J_1 = 17 Hz J_2 = 11 Hz). MS *m/e* (rel. int.) 306 (0.5) 291 (0.1), 288 (1.5), 178 (16), 177 (100), 176 (11), 137 (12.5), 121 (18), 109 (9).

Dehydration of **17**. To a stirred soln of **17** (360 mg) in pyridine (2 ml) at 0°, under N_2, $POCl_3$ (0.4 ml) was added. After 6 h at 25° working as is customary, by chromatographie and plc, it affords **5** (13 mg), identical to the natural product.

Ethyl trixagoyl malonate **2b** was eluted with hexane–ether (98:2) IR (ν cm^{-1}) 3040, 1750, 1730, 1660, 1640, 1140, 1030, 970, 880.

Methyl trixagoyl malonate **2c** eluted with hexane–ether (9:1) $[\alpha]_D$ +6.6° (c, 1.4, $CHCl_3$) (Found C 73.85 H 9.78) $C_{24}H_{38}O_4$ requires C 73.87 H. 9.74%) IR (ν cm^{-1}) 3060, 1760, 1740, 1675, 1640, 1275, 1090, 980, 895. MS *m/e* (rel. int.) 390 (0.5), 375 (1), 330 (2), 315 (2), 273 (8.5), 272 (21), 177 (96), 175 (79), 81 (100).

Isobutyl trixagoyl malonate **2d** was eluted with hexane–ether (9:1) $[\alpha]_D$ +1.0° (c, 0.98, $CHCl_3$) IR (ν cm^{-1}) 3060, 1760, 1740, 1665, 1640, 1330, 1275, 1150, 1020, 980, 890. MS *m/e* (rel. int.) 432 (0.1), 417 (0.5), 272 (21.5), 257 (24), 177 (44), 175 (100), 149 (59), 133 (79), 81 (74).

Saponification of **2d**. To **2d** (98 mg) 10% KOH MeOH (3 ml) was added and the mixture was left to stand at room temperature for 5 h; on normal work-up it afforded **1** (68 mg).

Ditrixagoyl malonate **2e** was eluted with hexane–ether (98:2) IR (ν cm^{-1}) 3090, 3040, 1770, 1750, 1660, 1640, 1280, 1160, 890.

Dinortrixagone **6** was eluted with hexane–ether (9:1) $[\alpha]_D$ +13.7° (c, 0.77, $CHCl_3$) UV (EtOH) 291 nm. IR (ν cm^{-1}) 3060, 3030, 3010, 1680, 1660, 1640, 1625, 1585, 1250, 970, 885. ^{1}H NMR (δ ppm) 0.83 (s, 3H), 0.91 (s, 3H), 1.86 (s, 3H), 2.14 (s, 3H), 4.49 and 4.70 (2s, 2H), 5.85 (d, 1H, J = 11 Hz), 5.91 (d, 1H, J = 15 Hz), 7.25 qu, 1H, J_1 = 11 Hz J_2 = 15 Hz). MS *m/e* (rel. int.) 260 (7), 245 (15.5), 227 (15), 171 (70.5), 170 (88), 161 (100), 159 (44), 121 (91), 109 (94).

6,7-*epoxy trixagol* **7a** was eluted with hexane–ether (8:2) IR (ν cm^{-1}) 3400, 3080, 1650, 1150, 1005, 895. Acetylation of **7a**: A soln of **7a** (40 mg) in Ac_2O (3 ml) and pyridine (3 ml) was left to stand at 25° for 2 h. The residue was extracted with ether and washed with 2*N* HCl and water. The combined ether extracts were dried over Na_2SO_4 and the solvent evaporated to obtain **7b**.

Trixagodiol A **8a** was eluted with hexane–ether (7:3) $[\alpha]_D$ +10.1° (c, 2.14, $CHCl_3$) IR (ν cm^{-1}) 3360, 3040, 3010, 1660, 1640, 1100, 1000, 970, 880. MS *m/e* (rel. int.) 288 (14), 273 (36), 255 (46), 203 (44), 177 (32), 161 (44), 121 (59), 109 (100), 81 (43).

Acetylation of **8a**. **8a** (80 mg) was acetylated with Ac_2O/Py at room temperature, to give **8b** (85 mg): IR (ν cm^{-1}) 3450, 3040, 1735, 1660, 1640, 1230, 1100, 1020, 970, 885. ^1H NMR (δ ppm) 0.85 (s, 3H), 0.91 (s, 3H), 1.20 (s, 3H), 1.69 (s, 3H), 1.98 (s, 3H), 2.71 (d, 2H, J = 4 Hz), 4.50 (d, 2H, J = 6 Hz), 4.54 and 4.73 (2s, 2H), 5.32 (t, 1H, J = 6 Hz), 5.48 (sAB, 2H, J = 4 Hz).

Dehydration of **8b**. To a stirred soln of **8b** (50 mg) in pyridine (1.2 ml) at 0° $POCl_3$ (0.4 ml) was added, under N_2, and the soln left to stand at room temperature for 3 h. After Ag^+ Si gel C, the product yielded **18** (17 mg). **18**: UV (EtOH) 230 nm IR (ν cm^{-1}) 3070, 3020, 1740, 1670, 1640, 1610, 1230, 1020, 970, 880, 850. ^1H NMR (δ ppm) 0.85 (s, 3H), 0.91 (s, 3H), 1.71 (s, 3H), 1.97 (s, 3H), 2.76 (d, 2H, J = 7 Hz), 482 (s, 2H), 5.50 (dt, 1H, J_1 = 16 Hz J_2 = 7 Hz), 6.00 (d, 1H, J = 16 Hz).

Trixagodiol B **9a** was eluted with hexane–ether (7:3) $[\alpha]_D$ + 7.4° (c, 1.92, $CHCl_3$) IR (ν cm^{-1}) 3330, 3060, 3010, 1660, 1640, 1050, 1000, 895, 850. MS *m/e* (rel. int.) 306 (5.5), 288 (29), 273 (51), 255 (53), 220 (32), 187 (62), 147 (64), 109 (100), 95 (73), 81 (49).

Acetylation of **9a**. **9a** (50 mg) was acetylated with Ac_2O/Py at 25° and gave **9b** (57 mg): IR (ν cm^{-1}) 3060, 3010, 1740, 1665, 1640, 1240, 1020, 950, 985. ^1H NMR (δ ppm) 0.85 (s, 3H), 0.92 (s, 3H), 1.71 (s, 3H), 1.95 (s, 3H), 1.97 (s, 3H), 4.47 (d, 2H, J = 7 Hz), 4.51 and 4.71 (2s, 2H), 4.72 and 4.93 (2s, 2H), 5.02 (m, 1H), 5.29 (t, 1H, J = 6 Hz).

Synthesis of **8a** *and* **9a** *by singlet oxygen oxidation of trixagol*. A solution of **1** (300 mg) *i*-PrOH (20 ml) and Rose bengal (7 mg) was stirred for 10 h exposed to diffuse sunlight, followed by reduction with $NaBH_4$ (300 mg) in MeOH (10 ml). When the MeOH had been evaporated the organic phase was extracted with ether three times. The combined ethereal soln was washed with water, dried over Na_2SO_4 and concentrated to yield 308 mg. On acetylation with Ac_2O/Py the product gives 320 mg which by C afford **8b** (64 mg) and **9b** (80 mg) whose saponification by KOH/MeOH gives **8a** (55 mg) and **9a** (60 mg) identical to the natural products.

Trixagodiol C **10a**. Was eluted with C_6H_6-AcOEt (8:2). IR (ν cm^{-1}) 3340, 3040, 3010, 1660, 1640, 1060, 990, 890, 850.

Acetylation of **10a**. **10a** (50 mg) was acetylated with Ac_2O/Py at room temperature, affording **10b** (59 mg). IR (ν cm^{-1}) 3070, 3020, 1740, 1670, 1640, 1240, 1030, 890, 850. ^1H NMR (δ ppm) 0.86 (s, 3H), 0.96 (s, 3H), 1.62 (s, 3H), 1.73 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 4.52 (d, 2H, J = 6 Hz), 4.57 and 4.78 (2s, 2H), 4.99 (t, 1H, J = 7 Hz), 5.10–5.50 (2t, 2H, J = 6 Hz). MS *m/e* (rel. int.) 390 (0.5), 321 (3.5), 315 (10), 270 (27), 255 (29.5), 203 (43), 202 (100), 187 (25), 147 (47), 133 (33), 81 (45).

Synthesis of **10a**. **7b** (530 mg) with anhyd. NaOAc (770 mg) in glacial AcOH (7 ml) were heated at 60–65° for 6 h. The product was extracted with ether and washed with $NaHCO_3$ aq. affording **11b** (330 mg) after chromatographical purification. 270 mg of **11b** dehydrated with $POCl_3$ by proceeding as before, afforded **9b** (60 mg) and **10b** (75 mg). Saponification of **10b** with KOH/MeOH gave **10a** identical to the natural product.

1,6-*diacetate of trixagotriol* **11b** was isolated in the previously acetylated fraction **7**, eluted with hexane–ether (8:2). IR (ν cm^{-1}) 3460, 3040, 3010, 1730, 1660, 1640, 1230, 1120, 1040, 1020, 950, 880. ^1H NMR (δ ppm) 0.86 (s, 3H), 0.91 (s, 3H), 1.20 (s, 3H), 1.71 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 4.56 (d, 2H, J = 6 Hz), 4.52 and 4.63 (2s, 2H), 4.60–4.80 (m, 1H), 5.28 (t, 1H, J = 6 Hz). MS *m/e* (rel. int.) 390 (1), 288 (4.5), 270 (4), 177 (100), 175 (38), 136 (52), 121 (73), 109 (71).

Trixagotriol **11a** Saponification with KOH/MeOH of **11b**, **11a** was isolated. To a soln of **11a** (180 mg), 2,2-dimethoxypropane (1.5 ml) and acetone (20 ml), 1 crystal of *p*-toluenesulphonic acid was added as catalyst. The reaction is instantaneous at room temperature. By plc, the reaction product eluted with hexane–ether (1:1) afforded **17** (23 mg). Synthesis of **11a**: **11a** was synthesized by saponification of **11b** obtained by acetolysis of **7b** (synthesis of **10a**). The saponification of **11b** (100 mg) with 10% KOH/MeOH (7 ml) proceeding as is customary afforded **11a** (65 mg), identical to the natural material.

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