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FURTHER STUDIES ON HYDROXYPEREZONE DERIVATIVES[a]

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Abstract—Hydroxyperezone monoangelate (**2c**) was transformed into the mixture of α-(**8**) and β-perezols (**9**). Separation of these compounds permitted stereochemical assignments by NMR and ORD. The position of the angeloyl moiety in **2c** was independently tested by its conversion to O-methylhydroxyperezone (**1d**) identified with a sample synthesised from perezone (**1a**). Several reactions distinguished the natural hydroxyperezone (**2a**) from the synthetic hydroxyperezone hydrate (**3**).

In continuation of the studies of perezone (**1a**) and their derivatives§ in 1852,[1] we would like to report some aspects of the chemistry of these compounds. In an earlier communication[2] we described the isolation of terpenoids from an unidentified *Perezia* species and from *P. hebeclada*. We also suggested, based on biogenetical grounds, that the natural occurring monoesters of hydroxyperezone (**2a**) should have the esterifying group at the oxygen near the quinonoid Me group, as shown in **2b** and **2c**. The chemical evidence required to confirm this suggestion for the particular case of hydroxyperezone monoangelate are described, together with the crystalline derivatives of natural hydroxyperezone (**2a**) and of hydroxyperezone hydrate (**3**), which clearly distinguish between these two compounds.

The hexane extracts of a new supply of roots of *Perezia sp.* from the local market, were chromatographed on silica gel, yielding a small amount of perezone (**1a**), which was identified by direct comparison with an authentic sample[3] and as the main constituent, a red oil b.p. 88° (1 mm) $(\alpha)_D$ −15° to which structure **2c** was ascribed in accordance with spectral properties. The IR spectrum shows OH (3330), ester CO (1740) and quinonoid ring (1655, 1640 and 1625 cm^{-1}) absorptions, while in the PMR spectrum both the typical signals of an angeloyl ester[4] and those corresponding to hydroxyperezone (**2a**) could be recognized. The intensities of the PMR signals are in excellent agreement to a monoangelate composition.

Further proof of the structure of O-angeloylhydroxyprerzone (**2c**) was obtained by alkaline treatment which gave angelic acid, identified by comparison of the spectral properties with those of an authentic sample.[4]

The position of the angeloyl group was established by thermal treatment of **2c** which provided a mixture of the previously described[2] α-(**8**) and β-perezols (**9**). Although this rearrangement proceeded with only 10% yield while the perezone (**1a**)-pipitzols (**6**+**7**) transormation gives ~60%, it shows that when the quinonoid hydrogen of perezone (**1a**) is replaced by an angelate group the reaction can still occur. It is interesting to remember that both hydroxyperezone (**2a**) and its monoisovalerate (**2b**) failed[2] to undergo the transformation under the conditions which brought about the perezone (**1a**)-pipitzol (**6**+**8**) rearrangement.[5]

Successive careful TLC separations yielded for the first time pure samples of both α-(**8**) and β-perezols (**9**). The physical and spectral properties of these two compounds are summarized in the Experimental. Their stereochemistry was deduced from their PMR spectra, since the *gem*-dimethyl

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singlets of α-perezol (**8**) show a chemical shift difference of 0·12 ppm in contrast to 0·03 ppm found for α-pipitzol (**6**), while those of β-pipitzol (**7**) show only one singlet in contrast with the chemical shift difference of 0·03 ppm found in β-perezol (**9**). Furthermore, the secondary Me doublet of α-perezol (**8**) at 1·41 ppm is found 0·06 ppm lower field than that of β-perezol (**9**), in excellent agreement with the differences observed (0·07 ppm) for the same proton signals in α-(**6**) and β-pipitzols (**7**) which are located at 1·37 and 1·30 ppm respectively. These stereochemical assignments were confirmed by ORD measurements, since α-perezol (**8**) shows a positive Cotton effect of 20000° at 335 nm (α-pipitzol has[6] $(\phi)_{335}$ + 12500°) and β-perezol (**9**) a negative Cotton effect of 18400° at 340 nm (β-pipitzol (**7**) has[6] $(\phi)_{335}$ − 10100°).

Independent proof of the position of the angeloyl group in **2c** was obtained by preparing O-methylhydroxy-perezone (**1d**) from both angeloylhydroxyperezone (**2c**) and from perezone (**1a**). Treatment of **2c** with dimethylsulfate in the presence of potassium carbonate gave O-angeloyl, O'-methylhydroxyperezone whose PMR spectrum shows the disappearance of the very broad OH signal at 7·1 ppm and the presence of a OMe singlet at 3·99 ppm. Catalytic hydrogenation of the above oily material using 5% Pd-C, saturated the ester moiety without affecting the isopropenyl grouping of the side chain of the terpenoid, since the angeloyl proton at 6·25 ppm and the vinylic Me signals due to the side chain of the hydroxyperezone moiety remained unchanged. Vigorous alkaline treatment in heterophase, gave a violet alkaline fraction which after acidulation, extraction and chromatography yielded O-methylhydroxyperezone (**1d**). On the other hand, treatment of perezone (**1a**) with benzyl amine gave violet crystals of **1b** which show PMR signals at 7·29 ppm (5 aromatic protons) and 4·62 ppm (CH_2 group). They were methylated to yield **1c** as a violet oil (OMe singlet at 3·94 ppm). Hydrogenolysis of the above material followed by alkaline air oxidation, neutralization and chromatography, gave O-methylhydroxyperezone (**1d**) which was identical (IR, UV, PMR and TLC) with the sample obtained from angeloyhydroxyperezone (**2c**).

In order to establish definitely if synthetic hydroxyperezone has a molecule of water of crystallization as suggested by Kögl and Boer,[7] or if a chemical process gave a new terpenoid, several reactions were performed, which established that water added to the double bond of the side chain. Treatment of hydroxyperezone (**2a**) with Ac_2O–NaOAc in the presence of zinc dust afforded the tetraester (**4**) while the same reaction performed on **3** yielded the pentaester **5b**. The structures of **4** and **5b** were assigned in agreement with $C_{23}H_{30}O_8$ and $C_{25}H_{34}O_{10}$ (analysis) respectively and both show IR absorptions corresponding to aromatic acetate groupings at 1782 cm^{-1} in addition to the aliphatic acetate band at 1823 cm^{-1} found in **5b**. The PMR spectra are also consistent with these structures. Compound **4** shows a singlet (12H) for the four acetyl methyls and the isopropeny signals as in perezone (**1a**), while **5b** displays singlets at 2·30 and 2·28 ppm (6H each) due to the aromatic acetates, a singlet (3H) at 1·96 ppm due to the aliphatic acetate and a singlet at 1·36 ppm (6H) due to the *gem*-dimethyl protons.

Both **2a** and **3** when treated with Ac_2O–NaoAc under hydrogen atmosphere in the presence of Adams catalyst, gave the tetraester **5a** as deduced from the elemental composition $C_{23}H_{32}O_8$, the IR band at 1782 cm^{-1} and PMR signals at 2·26 (6H) and 0·82 (6H, doublet J = 6 Hz). This suggests that the side chain of **2a** was hydrogenated while the tertiary OH of **3** was lost either by hydrogenolysis or dehydration followed by hydrogenation of the double bond. A sample of **5a** was also obtained by catalytic hydrogenation of **4**. Furthermore, treatment of **2a** with aqueous acetic acid in the presence of a small amount of sulfuric acid gave **3** in good yield.

The difference between **2a** and **3** have previously[8] been observed by PMR and MS measurements and from the oily derivatives. However, the clearest spectral differences, were obtained by natural abundance C-13 NMR spectroscopy.[9] A typical sample obtained by hydrolysis of anilidoperezone was observed to be a mixture of *ca* 60% of **2a** and 40% of **3**. The signals due to the quinonoid ring and the secondary and aromatic Me groups show the same chemical shifts in both compounds, while in **2a** the sp^2 carbon resonances of the side chain appear at 124·3 and 130·2 ppm in contrast to 43·5 and 68·5 ppm shown for the new methylene and the carbon bearing the new OH group in **3**. The remaining CH and CH_2 signals show smaller chemical shift differences while the *gem*-dimethyl signals of the **2a** appear at 17·2 and 25·2 ppm and were shifted to 28·9 and 29·3 in **3**.

EXPERIMENTAL

M.ps are uncorrected, IR spectra in $CHCl_3$ on Perkin Elmer 421, 521 or 337, UV spectra in 95% EtOH on Unicam SP-800, PMR spectra in $CDCl_3$ with internal TMS in Varian Associates A-60, A60A or HA-100, CMR as previously described[9] rotations and ORD curves* on Perkin Elmer 141M and microanalysis by A. Bernhardt Laboratories, West Germany.

Extraction of Perezia sp. The dried and ground roots (400 g) were extracted twice with 1·5 l of hexane under reflux during 12 h. The combined extracts were evaporated to dryness under vacuum yielding 25 g of a red oil. A portion of this material was chromatographed on silica gel using a mixture of benzene-EtOAc (19:1) as the eluent. It

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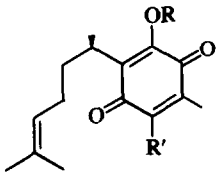
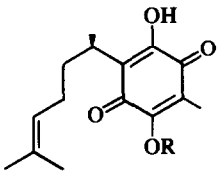


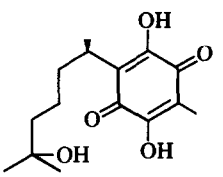
1

a: R = H; R′ = H
b: R = H; R′ = $NHCH_2Ph$
c: R = Me; R′ = $NHCH_2Ph$
d: R = Me; R′ = OH



2

a: R = H
b: R = $COCH_2iPr$
c: R = CO



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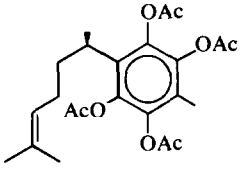

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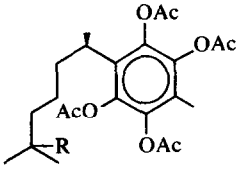
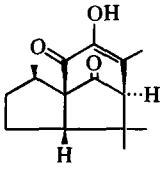


5

a: R = H
b: R = OAc



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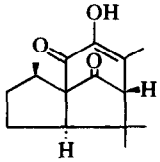

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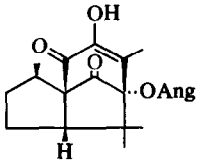

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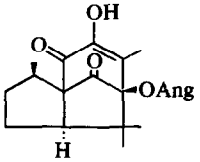

9

was found that the extract contains 12% of **1a** which was identical with an authentic specimen and 66% of **2c**. The pure red oil showed b.p. 88° (1 mm), $(\alpha)_D$ −15° (MeOH), λ_{max} 207, 222 (shoulder), 274 nm; ϵ, 27800, 18800, 10500; IR bands at 3330 (OH group), 1740 (ester CO group) and 1655, 1640 and 1625 cm^{-1} (quinonoid absorption bands; MS: *m/e* 346 (M^+) and *m/e* 83 (100% peak).

Alkaline treatment of hydroxyperezone angelate (**2c**). A soln of **2c** (280 mg) in MeOH (5 ml) was refluxed during 30 h in the presence of 50% NaOH (8 ml). The soln was acidified and steam distilled. The distillate was extracted with AcOEt, washed with water, dried and evaporated, yielding 58 mg of crystals m.p. 43–44°, which were identical with angelic acid.

Thermal treatment of **2c**. A sample of **2c** (11 g) was heated at 200° during 15 h. The dark paste was chromatographed on SiO_2 Grace grade 922, 200–325 mesh (300 g) using an hexane-benzene elution gradient. The fractions eluted with pure benzene crystalized. They were combined and recrystallized from acetone-hexane yielding 1 g of the mixture of α-(**8**) and β-perezols (**9**), m.p. 151–152° which were identical with an authentic sample.

The isomer separation was carried out on TLC plates (Merck F-254, 2 mm) using benzene-EtOAc (19:1) as eluent. This operation was repeated 3 times until the separation was complete.

α-perezol (**8**) showed m.p. 155–157°; positive $FeCl_3$ test; λ_{max} 218 and 278 nm (ϵ, 13500, 9300); IR bands at 3460 (OH), 1780 (bridged cyclopentanone), 1735 (ester CO) and 1680 and 1650 cm^{-1} (enolized α-diketone); PMR singlets at 0·97, 1·08, and 1·92 doublet at 1·41 (J = 7 Hz), vinyl Me multiplet centered 1·98, angelate vinyl proton at 6·12 and OH signal at 6·10 ppm; ms: *m/e* 346 (M^+) and *m/e* 182 (100% peak); ORD (*c*, 0·003, dioxane): $(\phi)_{550}$ +1100°, $(\phi)_{350}$ +15200°, $(\phi)_{335}$ +20000°; $(\phi)_{330}$ +10300°, $(\phi)_{329}$ +3300°.

β-perezol (**9**) showed m.p. 140–142°; positive $FeCl_3$ test; λ_{max} 218 and 281 nm (ϵ, 12100, 8200); IR bands at 3480 (OH), 1785 (bridged cyclopentanone), 1735 (ester CO) and 1680 and 1655 cm^{-1} (enolized α-diketone); PMR singlets at 1·03, 1·06, 1·91, doublet at 1·35 (J = 7 Hz) vinyl Me multiplet centered at 2·01, angelate vinyl proton at 6·12 and OH signal at 6·06 ppm; MS: *m/e* 346 (M^+) and *m/e* 83 (100% peak); ord (*c*, 0·000625, dioxane): $(\phi)_{450}$ −2200°, $(\phi)_{350}$ −12100°, $(\phi)_{340}$ −18400°, $(\phi)_{330}$ −12600°, $(\phi)_{328}$ −9100°.

Treatment of perezone with benzyl amine. A soln of **1a** (1 g) in ether (50 ml) was refluxed during 2 h in the presence of benzylamine (0·5 g). The soln was washed with dil HCl and water, dried over Na_2SO_4 and concentrated. This yielded 1·1 g of **1b** which was recrystallized from ether-hexane to give violet crystals m.p. 120–121°, λ_{max} 213, 314 nm; ϵ, 20600, 12400, IR bands at 3320 (NH), 3250 (OH), 1640 and 1575 cm^{-1} (quinonoid ring) (Found: C, 74·62; H, 7·60; N, 3·97; O, 13·73. Calc. for $C_{22}H_{27}NO_3$: C, 74·71; H, 7·70; N, 3·96; O, 13·58%).

Methylation of **1b**. A soln of **1b** (1 g) in acetone (15 ml) was refluxed with K_2CO_3 (4 g) and Me_2SO_4 (1 g) during 2 h.

The soln was filtered, concentrated to a small volume and disolved in AcOEt. The organic layer was washed with 10% KOH and with water, dried and evaporated to dryness. This yielded 0·7 g of **1c** as a violet oil, which was further purified by distillation at 105° (0·25 mm Hg). It showed λ_{max}, 213, 305 nm; ϵ, 19400, 8900 and IR bands at 3330 (NH), 1650 and 1600 cm^{-1} (quinonoid ring).

Hydrogenolysis of **1c**. A soln of **1c** (0·5 g) in EtOH (30 ml) was hydrogenated in the presence of 50 mg of prehydrogenated 5% Pd/C until the absorption of H_2 ceased. The catalyst was filtered off and the soln evaporated to dryness. The residue was disolved in AcOEt and extracted with 15% KOH aq. The alkaline extract was acidified with HCl and reextracted with AcOEt. The organic layer was washed twice with water, dried and evaporated to dryness. The residue was chromatographed over SiO_2. The fractions eluted with benzene were combined yielding 50 mg of **1d** as an orange oil, λ_{max} 207, 223 (sho), 286 nm; ϵ 28500, 16700, 12800 IR bands at 3370 (OH), 1650 and 1630 cm^{-1} (quinonoid ring).

O-*Methyl hydroxyperezone* (**1d**). A sample of **22c** (1 g) was methylated with Me_2SO_4 and worked up as previously. The oily residue showed λ_{max} 207, 223 (sho) 285 nm; ϵ, 33700, 19600, 13300; and IR bands at 1650 and 1625 cm^{-1} (quinonoid bands) in agreement with the structure of O'-angeloyl, O-methyl hydroxyperezone. It was hydrogenated using 5% Pd/C as previously and the crude residue extracted vigorously with 15% KOH. The violet alkaline soln was acidified, extracted, dried, evaporated and chromatographed, yielding 100 mg of **1d** which were identical with the product obtained from **1a** by IR, UV, PMR and TLC.

Pentaester (**5b**). A soln of **3** (300 mg) in Ac_2O (10 ml) containing AcONa (60 mg) was refluxed (1 h) in the presence of Zn dust (600 mg). The Zn was removed by filtration and washed with hot Ac_2O. The combined Ac_2O was removed under vacuum and the residue disolved in ether was washed several times with water, dried over Na_2SO_4 and concentrated. Crystallization from ether-pentane gave 400 mg of **5b** as white needles m.p. 84–84°. The analytical sample obtained from the same solvents showed m.p. 86–87°, λ_{max} 207, 215 (sho) 234, 278, 324 nm; ϵ, 17150, 13350, 1450, 500, 600, IR bands at 1782 (aromatic acetates) and 1723 cm^{-1} (aliphatic acetate). (Found: C, 60·91; H, 6·95; O, 32·21. calc. for $C_{25}H_{34}O_{10}$: C, 60·72; H, 6·93; O, 32·35%).

Tetraester (**4**). A sample of **2a** (300 mg) was treated as in the previous case yielding 500 mg of **4** as white needles m.p. 112–113°. The analytical sample obtained from acetone-hexane showed m.p. 113–114°, λ_{max} 207 (sho) 209, 215 (sho) 232, 279, 310 nm; ϵ, 17350, 18000, 13950, 18000, 350, 450, IR band at 1782 cm^{-1} (aromatic acetates). (Found: C, 63·10; H, 6·99; O, 30·01. calc. for $C_{23}H_{30}O_8$: C, 63·58; H, 6·96; O, 29·46%).

Tetraester (**5a**). A soln of **3** (100 mg) in Ac_2O (20 ml) was hydrogenated over prehydrogenated Adams catalyst (15 mg) until the uptake of H_2 ceased. After addition of AcONa (100 mg) the mixture was stirred for an additional 24 h under H_2. The catalyst was filtered off and the solvent evaporated in *vacuo*. The residue was disolved in ether, washed with water, dried and evaporated. The residue crystallized from ether-pentane yielding 120 mg of **5a** as white plates m.p. 128–129°. The analytical sample obtained from the same solvents showed m.p. 134–135°, λ_{max} 206 (sho), 209, 215 (sho) 230, 312 nm; ϵ, 13800, 14200, 11650, 1700, 300, IR band at 1782 cm^{-1} (aromatic acetates). (Found: C, 63·23; H, 7·29; O, 29·45 calc, for $C_{23}H_{32}O_8$: C, 63·29; H, 7·39, O, 29·32%). This compound was identical with a sample obtained by treatment of **2a** under exactly the same reaction conditions and to another sample obtained using the sequence perezone (**1a**), dihydroperezone, anilidodihydroperezone, dihydrohydroxyperezone given by Kögl and Boer,[7] followed by reductive acetylation as above.

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