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Structural Determination of Nootkatol, a New Sesquiterpene Isolated from *Alpinia oxyphylla* Miquel Possessing Calcium-Antagonistic Activity

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Abstract □ Nootkatol, a new sesquiterpene possessing calcium-antagonistic activity, was isolated from *Alpinia oxyphylla* Miquel and characterized as (2*R*,4*R*,5*S*,7*R*)-eremophil-1(10),11-dien-2-ol.

Keyphrases □ Nootkatol—isolation from *Alpinia oxyphylla* Miquel, calcium-antagonistic activity □ Calcium antagonists—isolation of nootkatol from *Alpinia oxyphylla* Miquel

In the course of our survey of various crude drugs prepared from plant materials for pharmacologically active metabolites, we have found that a methanolic extract of the fruit of *Alpinia oxyphylla* Miquel (Zingiberaceae) has calcium-antagonistic activity in the rabbit aorta. Other work (1–3) on calcium antagonists, such as verapamil and gallopamil, have revealed that such drugs selectively inhibit the potassium chloride-induced contraction of vascular smooth muscle. In this paper, we have isolated for the first time a substance possessing calcium-antagonistic activity from a plant source.

EXPERIMENTAL[1]

Method of the Bioassay—Rabbits (2–3 kg) were killed by cervical dislocation. The aortas (60 × 4 mm) were removed and cut into helical strips. The strips were mounted vertically in a 20-mL organ bath containing Krebs-Ringer bicarbonate solution of the following composition (mM concentration): NaCl, 120; KCl, 4.8; $CaCl_2$, 1.2; $MgSO_4 \cdot 7H_2O$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 25.2; and glucose, 5.8, pH 7.4. This was bubbled with a gas mixture of oxygen-carbon dioxide (95:5) and maintained at 37°C. A resting tension of 1 g was applied to the strips and tension changes were isometrically recorded with a force-displacement transducer.

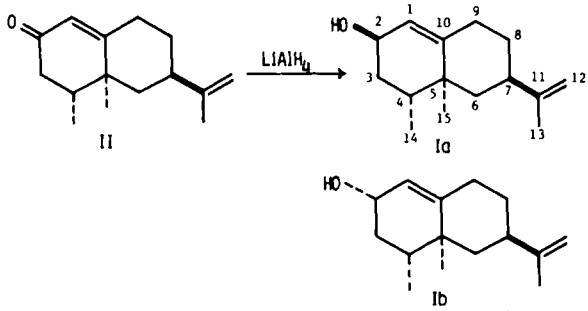
Isolation—The dried, powdered fruit[2] (10 kg) of *A. oxyphylla* Miquel were extracted three times with cold methanol (20 L). The methanolic extract was concentrated *in vacuo*, and the residue (1 kg) was partitioned between ethyl acetate and water. The aqueous layer was extracted with 1-butanol. The pharmacologically active ethyl acetate fraction (605 g) was chromatographed on silica gel[3] (benzene–ethyl acetate). The fractions containing the bioactive material [benzene–ethyl acetate (9:1)] were combined and further purified on a silica gel column with *n*-hexane–acetone (97:3) to afford 3.0 g of an active substance (Ia, 0.03% yield), named nootkatol, as colorless needles, mp 78–80°C (recrystallized from *n*-hexane), $[\alpha]_D$ + 208° (*c* 1.1 $CHCl_3$); IR (KBr): 3250 cm^{-1} (OH); MS: *m/z* 220 (M^+, 53%) and 177 (100); ^{1}H-NMR ($CDCl_3$): δ 0.89 (s, 3, C(5)—CH_3), 0.89 (d, 3, *J* = 6 Hz, C(4)—CH_3), 1.01 (t, 1, *J* = 13 Hz, C(6)—βH), 1.24 (dq, 1, *J* = 12 Hz and *J* = 4 Hz, C(8)—βH), 1.55 (m, 1, C(3)—βH), 1.63 (dt, 1, *J* = 13 Hz and *J* = 4 Hz, C(3)—αH), 1.71 (m, 3, C(11)—CH_3), 1.71 (m, 1, C(4)—βH), 1.79 (m, 1, C(8)—αH), 1.89 (dt, 1, *J* = 13 Hz and *J* = 3 Hz, C(6)—αH), 2.13 (m, 1, C(9)—βH), 2.23 (tt, 1, *J* = 13 Hz and *J* = 3 Hz, C(7)—H), 2.32 (m, 1, C(9)—αH), 4.06 (m, 1, C(2)—H), 4.68 (m, 2, C(12)—H_2), and 5.49 ppm (bd, 1, *J* = 5 Hz, C(1)—H); ^{13}C-NMR ($CDCl_3$): δ 15.21 (q, C-14), 16.83 (q, C-15), 20.79 (d, C-4), 32.54 (t, C-6*), 32.62 (t, C-8*), 34.98 (q, C-13), 36.21 (t, C-3), 38.32 (s, C-5), 40.72 (d, C-7), 44.60 (t, C-9), 64.37 (d, C-2), 108.71 (t, C-12), 121.73 (d, C-1), 148.55 (s, C-11), and 150.13 ppm (s, C-10). Assignments are interchangeable between carbon atoms with asterisks.

Anal.—Calc. for $C_{15}H_{24}O$: C, 81.76; H, 10.98. Found: C, 81.53; H, 11.28.

Reduction of Nootkatone (II)—To the ether solution (50 mL) of nootkatone[4] (1.9 g), 200 mg of $LiAlH_4$ was added and stirred overnight at room temperature. The suspension was treated as usual and extracted with ether. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel [*n*-hexane–acetone (97:3)]. Nootkatol (Ia) (40 mg) was eluted, followed by epinootkatol (Ib) (oily, 1980 mg). The aforementioned synthetic nootkatol was identical by IR, optical rotation, and melting point comparison with the material isolated from *A. oxyphylla*.

RESULTS AND DISCUSSION

From the fruit of *A. oxyphylla* Miquel, a calcium-antagonistic substance, named nootkatol, was isolated. In the mass spectrum the molecular ion (*m/z* 220) indicates a molecular formula of $C_{15}H_{24}O$. The single oxygen func-



Scheme I—Reduction of nootkatone (II) to nootkatol (Ia).

[1] Melting points were obtained on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotation was recorded on a Jasco DIP-180 digital polarimeter. IR spectra were obtained on a Shimadzu IR-27G photometer. ^{1}H-NMR spectra were recorded on a Nicolet NT-360 spectrometer. ^{13}C-NMR spectra were recorded on a Hitachi R-22 spectrometer. Mass spectra were obtained on a Shimadzu LKB-9000B.

[2] Purchased from Nippon Hunmatsu Yakuhin, Ltd., Osaka, Japan.

[3] Silica gel 60 (230–400 mesh); Merck.

[4] Supplied by Shiono Kohryo Co., Ltd., Osaka, Japan.

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tionality is ascribable to a hydroxyl group based on the absorption band at 3250 cm^{-1} in the IR spectrum. The ^{13}C-NMR signals at 108.71 (t), 121.73 (d), 148.55 (s), and 150.13 (s) ppm suggest that the molecule has two double bonds and, therefore, it appears to be bicyclic. Furthermore, the ^1H-NMR spectrum shows the presence of a tertiary methyl group at 0.89 (s, 3), a secondary methyl group at 0.89 (d, 3, J = 6 Hz), and isopropenyl groups at 1.71 (m, 3) and 4.68 ppm (m, 2). From the above data the structure of Ia appears to be similar to that of nootkatone (II), which has been isolated from *Chamaecyparis nootkatensis* (Lamb.)sprach and *Citrus paradisi* Macfad. (4–6). The main difference appears to be the presence of a hydroxyl group in the newly isolated material. The proton attached to the hydroxyl-bearing carbon is observed as a multiplet at 4.06 ppm in the ^1H-NMR spectrum, suggesting the hydroxyl group was β-oriented. To verify the proposed structure, II was reduced with $LiAlH_4$ in ether to yield two products (Scheme I). As expected, the minor one was identical with Ia on the basis of its melting point, IR spectrum, and optical rotation. Thus, Ia is characterized as (2*R*,4*R*,5*S*,7*R*)-eremophil-1(10),11-dien-2-ol.

It has been reported that in vascular smooth muscle, contractile response to potassium chloride is caused for the most part by increasing membrane permeability to Ca^{2+}, while the response to norepinephrine is caused mainly by releasing an intracellular pool of Ca^{2+} (7). Calcium antagonists such as verapamil and gallopamil have been shown to selectively inhibit the contractile activity produced by potassium chloride without having any effect on that induced by norepinephrine (8). In the present experiment, purified Ia (3×10^{-5} M) markedly inhibited the potassium chloride (40 mM)-induced contraction of the aorta, but did not have any effect on that produced by norepinephrine (10^{-6} M). The 50% inhibitory doses of Ia and verapamil for the potassium chloride (40 mM)-induced contraction were $\sim 10^{-5}$ and $\sim 10^{-7}$ M, respectively, indicating that the potency of Ia was approximately 100 times less than that of verapamil[5]. Furthermore, Ia (3×10^{-5} M) profoundly inhibited the increase in the $^{45}Ca^{2+}$ uptake of the aorta induced by potassium chloride[5]. These observations suggest that Ia specifically blocks the Ca^{2+}-influx into the muscle cell of the aorta.

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[5] Unpublished results.

Reverse-Phase Liquid Chromatography and Pharmacokinetic Study of Two Hydroxylated Analogues of Quinidine in Dogs

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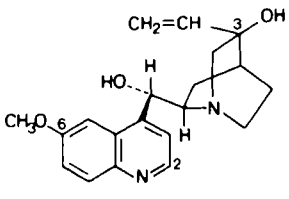
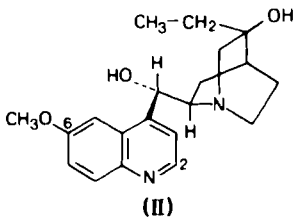
Abstract □ Two hydroxylated analogues of quinidine with antiarrhythmic properties, 3*S*-hydroxyquinidine and 3*R*-hydroxydihydroquinidine were assayed by reverse-phase high-performance liquid chromatography. The analytical technique uses plasma protein precipitation and direct injection on a C_{18} column, with an isocratic mobile phase and spectrofluorometric detection. 3*R*-Hydroxyquinidine is employed as the internal standard. Linearity is verified up to 5 mg/L for the two drugs; concentrations between 0.5 and 2.5 mg/L were measured with a *CV* of 0.5–2.07% for a given day and a sensitivity limit of 50 μg/L. Plasma concentration–time profiles and pharmacokinetic parameters in three dogs are presented after intravenous or oral administration. A significant difference is observed in terminal half-life, terminal rate constant, and total clearance of the two polar analogues of quinidine.

Keyphrases □ Quinidine analogues—hydroxylated, HPLC, pharmacokinetics in the dog □ Pharmacokinetics—hydroxylated analogues of quinidine in the dog, HPLC

Some of the metabolites of quinidine, especially 3*S*-hydroxyquinidine (I), 2′-oxoquinidine, and *O*-demethylquinidine, possess antiarrhythmic activity in mice and rabbits (1) and possibly in humans (2). Other analogues of quinidine, derived from *O*-demethylquinidine (3–6) or 7′-trifluoromethyldihydrocinchonidine (7), have recently been tested. Preliminary studies of I and 3*R*-hydroxydihydroquinidine (II) (8) indicate pharmacological activity of these two compounds when tested against chloroform-induced ventricular fibrillation and aconitine-induced arrhythmia in mice and rats or against coronary ligature in dogs.



(I)



(II)

This report describes a rapid, specific, accurate, and sensitive high-performance liquid chromatographic (HPLC) method for the determination of I and II in plasma. The procedure involves a modification of a recently published reverse-phase HPLC method for quinidine (9) using 3*R*-hydroxyquinidine as internal standard, with an isocratic mobile phase of acetonitrile–acetic acid–water and sensitive spectrofluorometric detection. Preliminary pharmacokinetic parameters of these two hydroxylated analogues of quinidine in the dog are reported.

EXPERIMENTAL

Reagents and Standards—All solvents used were analytical reagent grade[1]: acetonitrile for spectrometry, methanol, and 100% acetic acid. A phosphate buffer (0.05 M, pH 7.4) was prepared in distilled water. Pure standards of

[1] E. Merck, Darmstadt, West Germany.

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