Journal of Natural Products
Vol. 53, No. 6, pp. 1572-1574, Nov-Dec 1990

PSEUDOSEMIGLABRIN, A PLATELET AGGREGATION INHIBITOR FROM *TEPHROSIA SEMIGLABRA*[1]

LYDIA T. JONATHAN,[2] MESSANVI GBEASSOR,[3] CHUN-TAO CHE,*
HARRY H.S. FONG, NORMAN R. FARNSWORTH,

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy

GUY C. LE BRETON,

Department of Pharmacology, College of Medicine

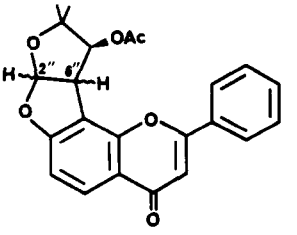
and DUANE L. VENTON

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60680

ABSTRACT.—Following bioassay-directed fractionation procedures, two 7-oxygenated-8-prenylflavones, (−)-pseudosemiglabrin [**1**] and (−)-semiglabrin [**2**], were isolated from an MeOH extract of *Tephrosia semiglabra*. (−)-Pseudosemiglabrin [**1**] displayed in vitro inhibitory effects on human platelet aggregation.

Tephrosia semiglabra Sond. (Leguminosae), a subtropical plant widely distributed throughout the southern part of Africa, is used in folkloric medicine as a health food in Lesotho. A review of the literature indicated that more than 35 species of *Tephrosia* have been studied for their chemical composition. They are rich in flavonoid constituents, including the 7-oxygenated flavones bearing a C-8 prenyl unit (1–9). In the present study, an MeOH extract of the aerial and root parts of *T. semiglabra* was found to exhibit an inhibitory effect against human platelet aggregation when evaluated in an in vitro bioassay system using the thromboxane A_2 mimetic U46619 as an aggregating agent ($IC_{50} = 240$ μg/ml). Bioassay-directed fractionation of this extract resulted in the isolation of an active principle (−)-pseudosemiglabrin [**1**], together with its isomer (−)-semiglabrin [**2**].

Being isomeric at positions 2″ and 6″, (−)-pseudosemiglabrin [**1**] and (−)-semiglabrin [**2**] are identical in many spectroscopic properties, with the exception of their nmr spectra. Thus, the H-5″ in **1** (δ 5.58) displayed substantial coupling ($J = 8.5$ Hz) with H-6″ (δ 4.62), while no such coupling was observed in **2**. In addition, the acetyl signal in **1** was highly shielded (δ 1.5), contrasting markedly with the more typical value of δ 2.22 observed in **2**. In the ^{13}C-nmr spectrum, carbons of the bifurano ring were shielded in **1** when compared with those of **2**. These spectroscopic properties were in close agreement with those reported in the literature for (−)-pseudosemiglabrin and (−)-semiglabrin (5, 7–9). They have been found in several *Tephrosia* species, including *T. semiglabra* (2), *Tephrosia apollinea* (5,6,9), *Tephrosia nubica* (10), and *Tephrosia purpurea* (7,8). (−)-Semi-



1 2″α-H, 6″α-H
2 2″β-H, 6″β-H

[1]Results have been presented in part at the 30th Annual Meeting of the American Society of Pharmacognosy, San Juan, Puerto Rico, August 6–10, 1989.

[2]On leave from the Department of Chemistry, Faculty of Science, The National University of Lesotho, Lesotho.

[3]On leave from the Department of Physiology, School of Science, Université du Benin, Togo.

glabrin [**2**] has been shown to display weak antimalarial activity in vitro (11).

Human platelet aggregation was monitored in response to the thromboxane A_2 mimetic U46619 (1 μM) at each step in the fractionation procedure. The active principle, (−)-pseudosemiglabrin [**1**], inhibited U46619-induced aggregation by 85 ± 5% at a final concentration of 6.5 μg/ml, whereas (−)-semiglabrin [**2**] produced 70 ± 6% inhibition at a much higher dose (45 μg/ml). These findings indicated that **1** was approximately seven times more active than **2** in blocking aggregation induced by U46619. On the other hand, when ADP (10 μM) was used as the aggregating agent, neither **1** nor **2** inhibited ADP-induced aggregation at dose levels of 13 μg/ml and 44 μg/ml, respectively. When (−)-pseudosemiglabrin [**1**] was tested in a concentration-response study by using U46619, an IC_{50} value of 12.5 μM was estimated. This potency is comparable to that of 13-azaprostanoic acid, a thromboxane A_2 antagonist (12).

In the literature, a number of flavonoid compounds have been shown to affect platelet aggregation, and the mechanism for the antiplatelet effects of some simple flavonoids has been attributed to the inhibition of phosphodiesterase activity (13,14). Although the mechanism of action of (−)-pseudosemiglabrin [**1**] remains to be determined, it appears to involve some inhibitory selectivity for the thromboxane-induced platelet aggregation. The stereospecificity of these prenylflavones in relation to their bioactivity is of general interest.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Kofler hot-stage apparatus and were uncorrected. The uv and ir spectra were obtained with a Beckman DU-7 spectrophotometer and a Nicolet MX-1 interferometer, respectively. Optical rotation was taken with a Perkin Elmer 241 polarimeter. The ^1H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ on a Nicolet NMC-360 instrument, and the low resolution eims were obtained with a Varian MAT-112 double-focusing spectrometer operating at 70 eV. Compound U46619 was obtained from Upjohn Co.

PLANT MATERIAL.—The aerial and root parts of *T. semiglabra* were collected in Lesotho in 1987. Voucher specimens have been deposited at the Faculty of Science, The National University of Lesotho.

EXTRACTION AND FRACTIONATION.—The dried, pulverized plant material (1.83 kg) was exhaustively extracted with MeOH, and the MeOH residue (IC_{50} = 240 μg/ml in the platelet aggregation inhibition assay) was washed with petroleum ether, followed by partition between $CHCl_3$ and aqueous MeOH. The $CHCl_3$ fraction (26.5 g) showed positive bioassay results (IC_{50} = 70 μg/ml).

ISOLATION OF ACTIVE PRINCIPLE.—The $CHCl_3$ fraction (25 g) was separated on Si gel by using the vacuum liquid chromatography technique on a sintered-glass funnel (15). Solvent mixtures of petroleum ether, $CHCl_3$, and MeOH were used in sequence of increasing polarities. Fifteen fractions (100 ml each) were collected, and those showing similar tlc profiles on Si gel G plates [$CHCl_3$-MeOH (10:1)] were pooled to give three combined fractions. Bioassay results indicated an enrichment of activity in the combined fraction 2 (IC_{50} = 63 μg/ml).

This active fraction (10 g) was then subjected to flash cc by gradient elution, starting with petroleum ether-$CHCl_3$ (10:2), followed by increasing amounts of $CHCl_3$ and MeOH. A total of 50 fractions (100 ml each) were collected. Fractions 8–12 were purified by preparative tlc [$CHCl_3$-hexane-MeOH (10:3:1)] to afford a white powder (1.56 g) displaying platelet aggregation inhibitory activity (IC_{50} = 10 μg/ml). Although tlc analysis of the substance in several solvent systems revealed a single fluorescent spot on the chromatogram, its ^1H-nmr spectra indicated the presence of two closely related compounds. Subsequently component **1** (35 mg, mp 173–175°, $[\alpha]_D$ −318°) was separated, with some difficulty, by fractional crystallization in a mixture of Et_2O and MeOH. The mother liquor contained a mixture of **1** and **2**.

Fractions 13–22 from the flash column also showed a similar fluorescent spot in tlc. They were therefore combined and treated with a hexane/MeOH mixture to yield a white solid, which was further purified by preparative tlc and crystallization to afford an analytically pure sample of **2** (23 mg, mp 248–250°, $[\alpha]_D$ −263°).

Compounds **1** and **2** were identified as (−)-pseudosemiglabrin and (−)-semiglabrin, respectively, on the basis of a comparison of their physical and spectral (uv, ^1H and ^{13}C nmr, ms) data with those reported in the literature (5, 7–9).

BIOASSAY FOR INHIBITORY ACTIVITY

AGAINST HUMAN PLATELET AGGREGATION.—Blood was obtained by venipuncture from healthy donors who had not received medication in 10 days. The blood was collected in a solution of citrate-phosphate-dextrose-adenine (CPDA-1), and platelet-rich plasma was prepared by contrifugation at 800 g for 20 min. Platelet aggregation changes were measured by the turbidometric method of Born (16) with a Chronolog lumiaggregometer. The agonist concentrations (U46619 at 1 μM or ADP at 10 μM) were titrated to yield maximal aggregation. Compound **1** was dissolved in 95% EtOH (13.2 mg/ml) and maintained at room temperature. Due to limited solubility, compound **2** was prepared at a lower concentration (8.8 mg/ml) and maintained at 65°. Test samples (or EtOH vehicle) were added at 1 min prior to the addition of aggregating agent. All samples were prepared in triplicate at each concentration, and the concentration-response curves were plotted by linear regression method.

ACKNOWLEDGMENTS

Two of us (L.T.J. and M.G.) were Fulbright Scholars (1987–88). This work was supported in part by a grant-in-aid from the American Heart Association (Chicago Office) to G.C.L. We are grateful to Mr. A. Amarquaye for his assistance in obtaining nmr and ms data. The Research Resources Center of the University of Illinois at Chicago is acknowledged for the provision of nmr and ms facilities during the course of this study.

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Received 19 February 1990