Structure Elucidation and Synthesis of a New Bioactive Quinazolone Derivative Obtained from *Glycosmis Cf. Chlorosperma*

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A new quinazolone derivative, (*Z*)-3,4-dihydro-3-(2-phenylethenyl)-quinazolin-4-one, was isolated from *Glycosmis cf. chlorosperma* SPRENG. (Rutaceae). The structure was elucidated on the basis of spectroscopic data and confirmed by comparison with synthetically obtained material. The compound showed bioactivity against different test organisms.

Key words *Glycosmis*; Rutaceae; secondary natural product; quinazolone; synthesis; structure elucidation

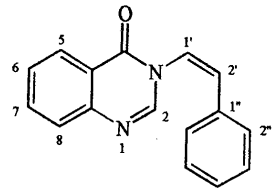
In connection with our search for bioactive compounds from the tropical Rutaceae of south and southeast Asia[1—4] we have investigated the chloroform fractions of a methanolic leaf extract of *Glycosmis cf. chlorosperma* SPRENG. (Java, Indonesia) from which we obtained, among other substances typical for *Glycosmis* (*e.g.* sulfur containing amides[2,4]), 5 mg of a compound with unusual UV/VIS and IR signal patterns.

The substance, which was named bogorin,[5] had a molecular formula of $C_{16}H_{12}N_2O$, as determined by HR-MS (Calcd 248.094, Found 248.095). The ^{13}C-NMR spectrum revealed that the molecular skeleton consisted of sp^2-hybridized atoms only. The ^{1}H-NMR spectrum showed three spin systems of five, four, and two protons respectively, and one additional singlet signal at δ=7.79 ppm (Table 1, $CDCl_3$). The four proton system results from an *ortho*-disubstituted aromatic ring, and the five proton system from a phenyl ring. The quinazolone system was determined from the fragmentation pattern of the mass spectrum (*m/z*=146, $M^+-C_8H_7$, *m/z*=119, $M^+-C_8H_7-HCN$)[6] and the sharp ^{1}H-NMR singlet resonance (H-2). The two spin system (δ_1=6.69 ppm, δ_2=6.94 ppm, J=9.2 Hz) indicated the presence of an isolated double bond. Its (*Z*)-configuration was confirmed by nuclear Overhauser effect (NOE) experiments. The double bond links the phenyl ring with the quinazolone skeleton (NOEs from 2′ to 2″ and 1′ to 2). The substitution was presumed to take place at position 3, because a comparison of the ^{13}C-NMR shift values of C-5 to C-8 with model compounds[7] suggested the nitrogen at position 1 to be aromatic.

Since the amount of natural product **5** was too small for spectroscopic characterization by two dimensional (2D)-NMR experiments and for bioactivity tests, a synthesis of bogorin was performed (Chart 1). The starting material, quinazolone (**1**) was prepared from anthranilic acid and formamide.[8] The phenylethenyl moiety was introduced by addition of styrene oxide.[9] Subsequent halogenation (thionyl chloride)[9] and dehydrohalogenation with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) exclusively gave (*E*)-bogorin (**4**) in about 25% overall yield. Photoinduced isomerization[10] of **4** yielded a 1 : 1 mixture of (*E*)- and (*Z*)-bogorin (**4**)/(**5**) which was readily separable by MPLC.

The ^{1}H- and ^{13}C-NMR spectra of the synthetic compound **5** were in full agreement with the signals for natural bogorin (Table 1). A second compound, which was obtained as a trace component in some bogorin preparations from natural sources, could be assigned as (*E*)-bogorin (**4**) by comparison of the ^{1}H-NMR spectra.

Bogorin (**5**) displayed antifungal activity in a bioautography assay[11] (*Cladosporium herbarium*, IC_{50}=40 μg/ml) as well as moderate cytotoxic activity against *Artemia salina*.[12] The (*E*)-isomer **4** and the synthetic precursors **1**—**3** had significantly lower activities.



(*Z*)-Bogorin (**5**)

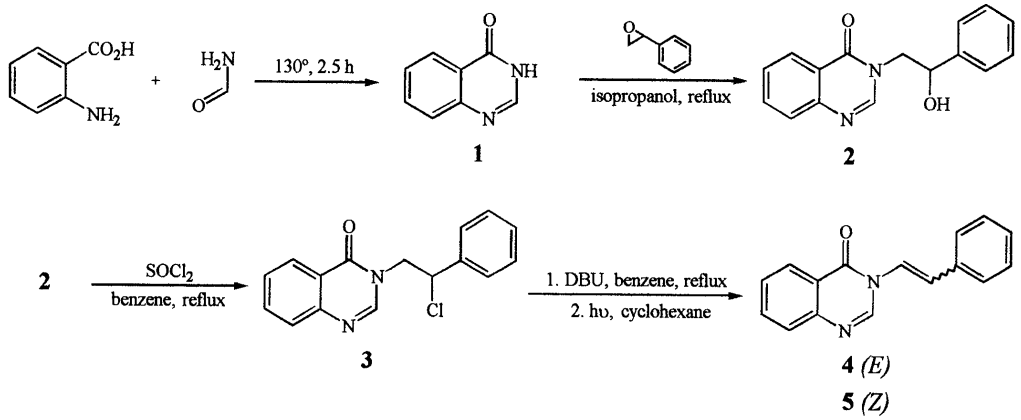


Chart 1

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Table 1. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) Data of Compounds **2**—**5**[a)]

	2	**3**	**4**	**5**	**5**[b)]	**2**	**3**	**4**	**5**	**5**[b)]
2	8.21 s	8.23 br s	8.63 s	7.83 s	7.79 s	149.2 d	148.4 d	144.7 d	146.8 d	146.2 d
4	—	—	—	—	—	161.7 s	161.4 s	160.2 s	161.1 s	161.6 s
4a	—	—	—	—	—	123.1 s	122.3 s	122.6 s	123.1 s	122.7 s
5	8.25 dd	8.25 dd	8.27 dd	8.27 dd	8.38 dd	127.1 d*	127.1 d*	127.5 d	127.2 d	127.6 d
6	7.54 ddd	7.57 ddd	7.59 ddd	7.58 ddd	7.54 ddd	127.5 d*	127.9 d*	128.3 d	128.1 d	128.2 d
7	7.81 ddd	7.83 ddd	7.85 ddd	7.84 ddd	7.78 dd	134.8 d	135.2 d	135.3 d	135.3 d	135.3 d
8	7.67 br d	7.67 br d	7.73 br d	7.65 br d	7.68 br d	128.3 d*	128.3 d*	128.5 d	128.5 d	128.4 d
8a	—	—	—	—	—	149.5 s	149.1 s	148.4 s	148.9 s	148.3 s
1′	4.45/3.93 dd	4.63/4.58 dd	7.87 d	6.94 d	6.94 d	54.9 t	54.4 t	124.4 d	125.4 d	124.4 d
2′	5.12 dd	5.56 dd	7.24 d	6.78 d	6.69 d	71.5 d	60.6 d	123.4 d	127.1 d	127.3 d
1″	—	—	—	—	—	143.2 s	139.1 s	136.0 s	134.3 s	133.4 s
2″/6″	7.51 br d	7.59 dm	7.61 dm	7.25—	7.19 m	126.8 d	128.3 d	127.5 d	129.6 d	129.4 d
3″/5″	7.39 br d	7.44 tm	7.42 tm	7.35	7.25—7.30	129.2 d	129.8 d	129.7 d	129.7 d	129.8 d
4″	7.31 tt	7.40 tm	7.34 tt	5H, m	3H, m	128.4 d*	130.0 d*	129.0 d	129.2 d	129.3 d

a) If not otherwise stated, measurements in acetone-d_6 at 300 K; ^{13}C data: denoted values (*) may be interchanged, assignments for compounds **4** and **5** are based on C, H-COSY; *b*) in $CDCl_3$. Coupling constants. **2**—**5**: $J(5,6)=8.0$ Hz, $J(6,7)=7.2$ Hz, $J(7,8)=8.3$ Hz, $J(5,7)=1.5$ Hz, $J(6,8)=1.2$ Hz, $J(2'',3'')=J(5'',6'')\sim J(3'',4'')=J(4'',5'')\sim 7.4$ Hz, $J(2'',4'')=J(4'',6'')=1.3$ Hz; **2**: $J(1'a,1'b)=13.5$ Hz, $J(1'a,2')=3.2$ Hz, $J(1'b,2')=9.2$ Hz; **3**: $J(1'a,1'b)=14.0$ Hz, $J(1'a,2')=5.8$ Hz, $J(1'b,2')=8.6$ Hz; **4**: $J(1',2')=15.0$ Hz; **5**: $J(1',2')=9.2$ Hz.

Furthermore, it should be noted that the structure of bogorin fits the common biosynthetic pathway considerations for substituted quinazolone alkaloids, *e.g.* indolopyridoquinazolones.[13)] These alkaloids are considered to be formed *via* anthranilic acid, a suitable amino acid derivative, and a C-1 building block (*e.g.* from methionine formate[13)]). In the case of bogorin, alkylation at the nitrogen atom N-3 has occured with a phenylalanine derived moiety.

Experimental

General NMR: Bruker AM 400 WB; MS: Finnigan MAT 900 S; IR: Perkin-Elmer 16 PC FT-IR; UV: Perkin-Elmer Lambda 5; HPLC: Hewlett-Packard HP 1090 II, UV diode array detection, column 290×4 mm (Spherisorb octadecyl silica (ODS), 5 μm), mobile phase MeOH (gradient 60—100%) in aqueous buffer (0.015 M phosphoric acid, 0.0015 M tetrabutylammonium hydroxide, pH=3), flow rate 1 ml/min; all steps of the isolation procedure and the purity of the final products were examined by HPLC.

Plant Material Java, Indonesia. Voucher specimens are deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

Extraction and Isolation Dried leaves (10 g) were extracted with MeOH at room temperature for 7 d, filtered and concentrated. The remaining aqueous phase was extracted with $CHCl_3$. The resulting extract was evaporated to dryness (150 mg) and roughly separated on a silica gel column (Merck Silica gel 60, 35—70 mesh) by elution with petroleum ether/ether mixtures with ether increasing from 0 to 100% and finally with 0—40% methanol in ether. The combined fractions eluted with 10—20% MeOH in Et_2O (65 mg) were separated by preparative MPLC with 20% EtOAc in hexane (400×40 mm column, Merck LiChroprep Silica 60, 25—40 μm, UV detection at 254 nm) to give 5 mg of pure (*Z*)-bogorin (**5**) and a further fraction of 3 mg containing traces of the (*E*) isomer (**4**). The spectroscopic properties of the isolated product **5** were identical with the synthetic compound **5** (see below). **5**: Colorless crystals, mp 73—76 °C. ^{1}H- and ^{13}C-NMR see Table 1. IR (CCl_4) cm^{-1}: 1700 s, 1610 s, 700 s. UV λ_{max} (EtOH) nm (ε): 310 (3.12), 269 (3.45), 227 (3.81). MS *m/z* (rel. int. %) 248 (97, M^+), 219 (15), 171 (25), 146 (100, quinazolone$^+$), 119 (30, 146−HCN), 102 (92, M^+-146), 77 (67, Ph^+). High resolution MS: Calcd for $C_{16}H_{12}N_2O$ 248.094, Found 248.095.

Synthesis of (*Z*)-Bogorin (5). 3,4-Dihydroquinazolin-4-one (1) Compound (**1**) was synthesized according to ref.[8)] 54.79 g (0.40 mol) of anthranilic acid was added in portions to 26.4 ml (0.66 mol) of formamide during heating of the resulting mixture to 130 °C (oil bath). At this temperature the mixture was a brown viscous liquid which changed to a hard crystalline cake after 2.5 h reaction time. The cake was crushed, treated with a little ethanol, and filtered. The crude product was recrystallized from 500 ml ethanol to give after washing with ethanol and drying (vacuum, 6 h) 48.6 g (83% th., 96% lit.) of compound **1** with mp 215—217 °C (ref.[8)] 215—216 °C).

3-(2-Hydroxy-2-phenylethyl)-3,4-dihydroquinazolin-4-one (2) Compound (**2**) was prepared modifying the general procedure given in ref.[9)] 11.0 g (0.075 mol) of 4-quinazolone (**1**) was suspended in 90 ml isopropanol containing 0.5 ml pyridine. This mixture was heated slowly to 120 °C (oil bath) and during this time 10 ml (0.088 mol) of styrene oxide was added. After dissolution of the components the reaction mixture turned from yellow to brown. After 5 h at reflux, the reaction mixture was allowed to cool slowly on the oil bath. 7.4 g of crystals formed within 6 h, after 2 weeks in the refrigerator a further 1.2 g could be collected. Total yield 8.6 g (43% th.) of slightly yellow crystalline material, mp 165—169 °C (ref.[9)] 166—168 °C). ^{1}H- and ^{13}C-NMR see Table 1. MS *m/z* (rel. int. %) 266 (12, M^+), 248 (8, M^+-H_2O), 160 (100, *N*-methylquinazolone$^+$), 147 (99, quinazolone·H^+), 132 (87), 130 (53), 120 (36), 119 (39, M^+-147), 107 (66), 102 (32), 79 (69), 77 (85), 51 (25).

3-(2-Chloro-2-phenylethyl)-3,4-dihydroquinazolin-4-one (3) 6.0 g (0.023 mol) of hydroxy compound **2** was added in portions to 2 ml (0.027 mol) thionyl chloride in 50 ml absolute benzene (rapid HCl and SO_2 evolution). After 1 h at reflux, the product was precipitated by addition of 400 ml ether and the mixture was left overnight in the refrigerator. The product (6.1 g, 93% th.) was a microcrystalline powder with mp 140—150 °C (mainly free base; ref.[9)] 198—200 °C for the hydrochloride and no data for the free base). This material was used for the next step without further purification. ^{1}H- and ^{13}C-NMR see Table 1. MS *m/z* (rel. int. %) 284 (62, M^+), 249 (16, M^+-Cl), 248 (12, M^+-HCl), 159 (47, methylenequinazolone$^+$), 146 (100, quinazolone$^+$), 140 (25, isotope peak to 138), 138 (75, $M^+-146=CH_2$–CHCl–Ph), 130 (37), 120 (30), 103 (24, 138−Cl), 102 (20, 138−HCl), 79 (72).

(*E*)-3-(2-Phenylethenyl)-3,4-dihydroquinazolin-4-one (4) 3.0 g (0.0106 mmol) of compound **3** was filtered over Al_2O_3 to remove HCl (solvent EtOH). EtOH was evaporated and the oil (still containing some EtOH) was dissolved in 400 ml benzene. This mixture was heated to reflux and 10 ml (0.068 mmol) DBU was added dropwise. After 6—18 h, DBU was removed by extraction with dilute HCl. After washing the benzene phase with water, drying over $MgSO_4$, and evaporation of the solvent, 2.1 g of white crystalline product **4** remained. Further purification was achieved by either chromatography over a short SiO_2 column (petrol ether : ethyl acetate=3 : 1) or by crystallization from 800 ml petrol ether. Yield 1.7 g (65% th.), mp 154—157 °C. ^{1}H- and ^{13}C-NMR see Table 1. MS *m/z* (rel. int. %) 248 (88, M^+), 205 (18), 171 (20), 146 (100, quinazolone$^+$), 119 (22), 118 (24), 102 (91, M^+-146), 77 (54, Ph^+), 57 (58).

(*Z*)-3-(2-Phenylethenyl)-3,4-dihydroquinazolin-4-one (Bogorin, 5) 150 mg (0.6 mmol) **4** was dissolved in 400 ml cyclohexane and irradiated with a Hg high pressure lamp (TQ-150, Heraeus, Hanau, Germany) over a period of 2 h.[10)] After 1.5 h, photo equilibrium was obtained (checked by ^{1}H-NMR). The resulting 1 : 1 mixture of **4** and **5** was separated on a home-made preparative MPLC column with approximately 7000 theoretical plates (420×40 mm, Merck LiChroprep Silica 60, 25—40 μm) with petrol ether : ethyl acetate=3 : 1. The first fraction was 45 mg of pure *Z*-isomer **5** (>99%), the second fraction (60 mg) was a 1 : 1 isomeric mixture, and the third fraction (40 mg) was pure *E*-isomer **4**. The melting point and all spectroscopic data

of synthetic **5** agreed with the data for the biogenic compound **5** (see Table 1 and Extraction and Isolation).

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