Journal of Natural Products
Vol. 52, No. 3, pp. 629-633, May-Jun 1989

ISOLATION OF FLAVONOIDS AND A CHALCONE FROM *HELICHRYSUM ODORATISSIMUM* AND SYNTHESIS OF HELICHRYSETIN

LUC VAN PUYVELDE,* NORBERT DE KIMPE,*[1,2] JEAN COSTA,[3] VIATEUR MUNYJABO,[3]
SPECIOSA NYIRANKULIZA, ETIENNE HAKIZAMUNGU, and NICEAS SCHAMP[2]

Centre Universitaire de Recherche sur la Pharmacopée et la Médecine Traditionnelle (CURPHAMETRA), Université Nationale du Rwanda, B.P. 117, Butare, Rwanda

ABSTRACT.—3,5-Dihydroxy-6,7,8-trimethoxyflavone, 3-*O*-methylquercetin, and helichrysetin were isolated from the flowers of the Rwandese medicinal plant, *Helichrysum odoratissimum*. Because of inconsistencies in the mp of the latter chalcone, a synthesis of helichrysetin was developed. 3-*O*-Methylquercetin was shown to be an active principle as it displayed antimicrobial activity.

Helichrysum odoratissimum (L.) Less. (Asteraceae), formerly known as *Helichrysum hochstetteri* (Sch.-Bip. ex A. Rich.) Hook.f. and *Helichrysum hochstetteri* var. *scabrum* Moeser (1), is a widespread herb throughout intertropical and southern Africa where it is used to relieve abdominal pains, heartburn, coughs, colds, and wounds (2). In the native medicine of Rwanda, *H. odoratissimum* is used to treat female sterility, menstrual pain (3), and eczema and is known under the names of Umutamatama, Manayeze, Umunyarugabo, Umutaranuka, and Rukanjabyuma. In the course of systematic studies on biologically active substances from medicinal plants of Rwanda, antimicrobial activity was found in the MeOH extract of the flowers of *H. odoratissimum* (4). Fractionation led to the isolation of two flavonoids, namely 3,5-dihydroxy-6,7,8-trimethoxyflavone and 3-*O*-methylquercetin (with antimicrobial activity), and one chalcone, helichrysetin.

3,5-Dihydroxy-6,7,8-trimethoxyflavone, was isolated from the petroleum ether extract in 0.38% yield as yellow-brown crystals [mp 150°; lit. (9) mp 149–150°]. The mass spectrum showed a molecular ion $[M]^+$ at m/z 344 (100% peak). The 5-OH, 6,7-diOMe pattern succeeds from $[M]^+$ and $[M-15]^+$ where $[M]^+$ is the 100% peak (5). The 8-OMe compounds mostly have their base peak at $[M-15]^+$, which is not the case here (6). The ^1H-nmr data were identical with the data in the literature (9), while the ^{13}C-nmr data are given for the first time in the Experimental section. It was possible to attribute the ^{13}C-nmr values (reported here for the first time) of the flavonoid by combination of the values of galangin, i.e., 3,5,7-trihydroxyflavone (7), and 4′,5′-dihydroxy-3,3′,6,7,8-pentamethoxyflavone (6). 3,5-Dihydroxy-6,7,8-trimethoxyflavone was previously isolated from *Helichrysum arenarium* (L.) DC. (8) and *Helichrysum graveolens* MB. (9). 3,5-Dihydroxy-6,7,8-trimethoxyflavone was also isolated from *Artemisia ludoviciana* Nutt. (10), but the data (mp 228–229°; ^1H nmr) did not agree with our flavonoid; the flavonoid isolated from *A. ludoviciana* is probably an isomer.

Helichrysetin, 1-(2,4-dihyroxy-6-methoxyphenyl)-3-(4-hydroxyphenyl)-2-propen-1-one, was isolated from the MeOH extract in 0.48% yield as yellow-orange crystals (mp 248°). The ir, ^1H nmr, and ms data fully agreed with the

[1]Senior Research Associate (Onderzoeksleider) of the Belgian National Fund for Scientific Research.
[2]Laboratory of Organic Chemistry, Faculty of Agricultural Sciences, State University of Gent, Coupure Links 653, B-9000 Gent, Belgium.
[3]Département de Chimie, Faculté des Sciences, Université Nationale du Rwanda, B.P. 117, Butare, Rwanda.

data from the literature (15), but the ^{13}C nmr is reported here for the first time (see Experimental).

This chalcone was previously synthesized (11), but obscure melting point characteristics were reported, as the compound sintered at 198° and melted at 235°. In addition, a ^{14}C-labeled derivative was synthesized, namely 2′,4′,4-trihydroxy-6′-methoxychalcone-[methyl-^{14}C], but no spectrometric data were reported (12). Later, the chalcone received the name helichrysetin and was reported to melt at 328–330° (13). The chalcone was also synthesized in 1976, but the synthetic chalcone had mp 142–143° (14). More recently, the chalcone was isolated from *Helichrysum heterolasium* Hilliard as a yellow oil, but the spectral characteristics completely matched the data of our compound (15). Finally, the same chalcone (mp 240–241°) was recently isolated from the aerial parts of the Argentinian plant *Achyrocline flaccida* (Weinm.) DC., but no spectral data except the uv spectrum were reported (16). In addition, some related chalcone glucosides have been reported in the literature (17,18).

As many discrepancies exist concerning the melting point of the chalcone, a synthetic study was undertaken. Our natural and our synthetic chalcone, (identical in all aspects) showed the following detailed melting point characteristics: darkening (203–206°), weakening (209–210°), becoming sticky (229°), and melting, i.e., formation of blood-red liquid (248°).

Helichrysetin was synthesized in the following way. 1,3-Dihydroxy-5-methoxybenzene was condensed with MeCN to afford the corresponding ketimine hydrochloride, which was hydrolysed to give 2,4-dihydroxy-6-methoxyacetophenone [62%, mp 208°, lit. (19) mp 205–207°]. No aldol condensation could be observed between 2,4-dihydroxy-6-methoxyacetophenone and 4-hydroxybenzaldehyde under the diluted basic conditions most often used. Also, the attempted aldol condensation with excess pulverized KOH in DMF (20) at 100° for 2 h gave no chalcone. The method of Klinke and Gibian (21) utilizing 60% aqueous KOH and equimolecular amounts of aldehyde and ketone, which is especially useful for synthesis of polyhydroxy aldol compounds, gave starting material after 24 h at room temperature, while heating caused side reactions to occur. An improved procedure, using a threefold molar excess of the aldehyde in 60% aqueous KOH containing some EtOH, was developed (room temperature 14 days; 68%). When chromatographed over Si gel, as described for the natural product helichrysetin, the yellow chalcone isolated proved to be identical in all respects with the compound isolated from *H. odoratissimum*. The mp's of the natural and the synthetic material were both 248°. *Anal.* calcd C 67.13%, found C 67.34%; calcd H 4.92%, found H 4.82%.

3-*O*-Methylquercetin was isolated from the MeOH extract in 1.4% yield as green crystals (mp 276–277°). All spectral data (ir, ^{1}H nmr, ^{13}C nmr, ms) are in full agreement with the data in the literature.

The occurrence of flavonoids in plant species is widespread, as evidenced from a literature review (22). Some of the plant species in which 3-*O*-methylquercetin has been found include *Eupatorium capillifolium* (Lam.) Small and *Eupatorium perfolatium* L. (23), *Vernonia cinerea* (L.) Less. and *Vernonia patens* H.B.K. (24), *Gutierrezia grandis* S.P. Blake (25), and *Vernonia amygdalina* Del. (26).

Of the three products isolated, only 3-*O*-methylquercetin showed antimicrobial activity, which was determined by the liquid dilution method. The minimum inhibitory concentration (MIC) values were determined as the lowest concentration of the compound completely inhibiting macroscopic growth. A comparison was made between the

flavonoid, tetracycline hydrochloride, and nystatin (Table 1). 3-*O*-Methylquercetin showed limited activity against Gram-positive bacteria.

TABLE 1. Antimicrobial Activity of 3-*O*-Methylquercetin, Tetracycline Hydrochloride, and Nystatin.

Bacteria/Fungi	Compound		
	3-*O*-Methylquercetin	Tetracycline hydrochloride	Nystatin
Gram-negative bacteria	Minimum inhibitory concentration (μg/ml)		
Enterobacter cloaceae (ATCC 23355)	100	12.5	
Escherichia coli (ATCC 25922)	100	6.25	
Klebsiella pneumoniae (ATCC 13883)	100	12.5	
Proteus vulgaris (ATCC 27853)	100	25	
Pseudomonas aeruginosa (ATCC 27853)	100	100	
Pseudomonas solanacearum[a]	100	100	
Salmonella typhimurium (ATCC 14028)	50	12.5	
Serratia marcescens (ATCC 8100)	100	25	
Shigella dysenteriae[b]	100	0.78	
Gram-positive bacteria			
Bacillus subtilis (ATCC 6633)	50	3.12	
Mycobacterium smegmatis (ATCC 607)	100	100	
Staphylococcus aureus (ATCC 12228)	6.25	100	
Streptococcus pyogenes (ATCC 19651)	100	6.25	
Fungi[c]			
Aspergillus flavus	100		12.5
Candida albicans (CBS 562)	12.5		0.78
Epidermophyton flocosum	100		100
Microsporum canis	100		50
Paecelomyces	100		50

[a]Isolated at the Faculty of Agronomy, UNR, Butare, from potatoes.
[b]Isolated from a clinical sample at the Department of Microbiology of the Faculty of Medicine, UNR, Butare.
[c]Supplied by the Clinic of Dermatology of the Faculty of Medicine of the State University of Ghent.

EXPERIMENTAL

GENERAL METHODS.—^{1}H-nmr spectra were recorded with Varian T-60 (60 MHz) and Bruker WH-360 (360 MHz) nmr spectrometers. Ir spectra were measured with a Perkin-Elmer model 1310 spectrometer, and mass spectra were obtained from a Varian-MAT 112 mass spectrometer (70 eV). Melting points were determined on a Kofler hotstage and on a Electrothermal Melting apparatus.

PLANT MATERIAL.—Flowers of *H. odoratissimum* were collected in the prefecture of Butare (southwestern Rwanda) in August 1984. Voucher herbarium species are deposited in the Herbaria of the INRS (Institut National de Recherche Scientifique) at Butare. The air-dried and powdered flowers (300 g) were successively extracted in a percolator until exhaustion with petroleum ether (40–60°), $CHCl_3$, H_2O, and MeOH. The petroleum ether extract (24.4 g) and the MeOH extract (33.6 g), which showed antimicrobial activity, were further fractionated.

PETROLEUM ETHER EXTRACT.—The extract was redissolved in petroleum ether (100 ml) and extracted with MeOH-H_2O (9:1) (5 × 200 ml). The petroleum ether phase was then concentrated to 11.4 g under reduced pressure, and the MeOH was removed from the MeOH/H_2O extract under reduced pressure. The H_2O phase was extracted with $CHCl_3$ (5 × 300 ml) which gave, after evaporation of the $CHCl_3$, a residue of 10.8 g. A portion of this $CHCl_3$ extract (4.2 g) was absorbed on 20 g of Si gel and chromatographed on a Si gel column (230 g) (Riedel de Haën, 230–400 mesh) in *n*-hexane and eluted with an *n*-hexane/toluene/$CHCl_3$/EtOAc/MeOH gradient. 3,5-Dihydroxy-6,7,8-trimethoxyflavone was isolated from the toluene-$CHCl_3$ (3:7) fraction after preparative tlc (Merck, Si gel 60, 2 mm) using toluene-$CHCl_3$ (2:8) as the eluent (450 mg, yield 0.38% of dry plant) and recrystallized from $CHCl_3$/*n*-hexane to afford brown-yellow crystals, mp 151°. Eims (70 eV) *m/z* (%) $[M]^+$ 344 (100), 329 (85), 301 (28),

286 (12), 172 (12), 105 (30), 87 (15); ^{13}C nmr (20 MHz, DMSO-d_6) δ 61.4, 61.8, and 60.6 (each q, 3 × OMe), 127.6 (d, C-6'), 128.6 (d, C-5'), 130.2 (d, C-4'), 128.6 (d, C-3'), 127.6 (d, C-2'), 131.0 (s, C-1'), 105.7 (s, C-10), 144.5 (s, C-9), 132.6 (s, C-8), 152.4 (s, C-7), 135.1 (s, C-6), 147.5 (s, C-5), 177.0 (s, C-4), 137.3 (s, C-3), 145.8 (s, C-2).

MeOH EXTRACT.—The extract was redissolved in MeOH-H_2O (1:1) (400 ml) and extracted with $CHCl_3$ (5 × 500 ml), which gave, after evaporation under reduced pressure, a residue of 16 g. A portion of the $CHCl_3$ extract (11.5 g) was absorbed on Si gel (50 g) and chromatographed on a Si gel column (550 g) (Riedel de Haën, 230–400 mesh) in C_6H_6 and eluted with a C_6H_6/$CHCl_3$/EtOAc/MeOH gradient.

HELICHRYSETIN.—1-(2,4-Dihydroxy-6-methoxyphenyl)-3-(4-hydroxyphenyl)-2-propen-1-one was isolated from the $CHCl_3$-EtOAc (65:35) fraction (1036 mg, yield 0.48% of the dry plant) and was recrystallized from $CHCl_3$ to afford yellow-orange crystals, mp 248°. ^{13}C nmr (20 MHz, Me_2CO-d_6) δ 56.3 (q, OMe), 92.2 (d, C-5'), 97.0 (d, C-3'), 128.0 (s, C-1), 160.6 (s, C-4), 131.2 (d, C-2 and C-6), 116.8 (d, C-3 and C-5), 125.2 (d, C-α), 143.3 (d, C-β), 106.3 (s, C-1'), 165.7 and 164.2 (each s, C-2' and C-4'), 168.9 (s, C-6'), 193.1 (s, C=O).

3-*O*-METHYLQUERCETIN.—3-*O*-Methylquercetin was isolated from the $CHCl_3$-EtOAc (35:65) fraction (3.090 g, yield 1.4% of dry plant) and was recrystallized from $CHCl_3$ to afford green crystals, mp 276–277°. All data agreed with the spectral data from the literature (27).

SYNTHESIS OF HELICHRYSETIN.—1,3-Dihydroxy-5-methoxybenzene (5 g, 35 mmol) was converted into 2,4-dihydroxy-6-methoxyacetophenone according to a literature procedure (19), with yield 4.1 g (62%), mp 208° [lit. (19) mp 205–207°]. All spectra (^{1}H nmr, ^{13}C nmr, ir, ms) agreed with the structure. 2,4-Dihydroxy-6-methoxyacetophenone (0.36 g, 2 mmol) and 4-hydroxybenzaldehyde (0.72 g, 6 mmol) were dissolved in 5 ml 60% aqueous KOH. The homogeneous mixture was stirred in the dark (protection by aluminum foil) for 14 days. The brown reaction mixture was then poured into 50 ml of 0.5 N HCl. To this mixture was added dropwise 2 N HCl until no further precipitate was formed. The reaction mixture was left 2 h at room temperature after which the precipitate was collected. After drying under vacuum (0.01 mm Hg), 0.41 g of yellow-brown and pure chalcone (68%) was obtained. Then the chalcone was chromatographed over Si gel under the same conditions as described above for the natural product helichrysetin.

ANTIMICROBIAL ASSAY.—The media used for antimicrobial tests were Mueller-Hinton broth (Difco) for bacteria and yeasts and Sabouraud-Dextrose broth (Difco) for fungi. The bacteria were inoculated in a Mueller-Hinton broth and incubated for 24 h (48 h for *Mycobacterium smegmatis*) at 37°. These cultures were adjusted with physiological saline solution to match one-half of McFarland No. 1 standard to obtain approximately 10^6 cells/ml. The yeast was inoculated in a Mueller-Hinton broth and incubated for 48 h at 37°. This culture was adjusted in physiological saline solution with a photometer at 530 nm to obtain approximately 10^5 cells/ml. The fungi were inoculated into Petri dishes containing Sabouraud-Dextrose agar and incubated at 27° until complete growth covered the surface of the agar plate. This growth was suspended in a physiological saline solution and adjusted with a photometer at 530 nm to obtain approximately 10^5 cells/ml.

The antimicrobial activity was determined by the liquid dilution method. The active principles were first dissolved in MeOH (5 mg/ml), after which 0.08 ml of the solution was added to the first test tube containing 4.0 ml of the broth in order to obtain a concentration of 100 μg/ml. Further dilutions in the broth were prepared: 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 μg/ml.

Next, 0.1 ml of the standardized suspension of microorganisms was added to each sample. The samples with microorganisms were incubated as static cultures for 24–48 h at 37° for bacteria and yeast, and for 7 days at 27° for fungi. MIC values were determined at the lowest concentration of the compound completely inhibiting macroscopic growth. Tetracycline hydrochloride and nystatin were used as positive controls with a 2% MeOH solution as a negative control. Three series of determinations were run for each compound and microorganism.

LITERATURE CITED

1. G. Troupin, "Flore du Rwanda, Spermatophytes, Vol. III," Institut National de Recherche Scientifique, Butare, Rwanda, 1985, p. 608.
2. J.M. Watt and M.G. Breyer-Brandwijk, "The Medicinal and Poisonous Plants of Southern and Eastern Africa," 2nd ed., E. and S. Livingstone, Edinburgh and London, 1962, p. 239.
3. L. Van Puyvelde, S. Mukarugambwa, P.C. Rwangabo, M. Ngaboyisonga, and Runyinga-Barabwiliza, *Afr. Med.*, **16**, 531 (1977).
4. Y. Boily and L. Van Puyvelde, *J. Ethnopharmacol.*, **16**, 1 (1986).
5. M. Goudard, J. Favre-Bonvin, M. Strelis-

ky, M. Nogradi, and J. Chopin, *Phytochemistry*, **18**, 186 (1979).
6. J.W. Roitman and L.F. James, *Phytochemistry*, **24**, 835 (1985).
7. E. Wollenweber, in: "The Flavonoids: Advances in Research." Ed. by J.B. Harborne and T.J. Mabry, Chapman and Hall, London and New York, 1982, pp. 189-259.
8. R. Hänsel, H. Rimpler, and R. Schwartz, *Tetrahedron Lett.*, 735 (1967).
9. R. Hänsel and B. Cubuken, *Phytochemistry*, **11**, 2632 (1972).
10. X.A. Dominguez and E. Cardenas, *Phytochemistry*, **14**, 2511 (1975).
11. G. Zemplen, A. Bognar, and K. Thiele, *Chem. Ber.*, **77B**, 446 (1944).
12. P.M. Dewick, *Phytochemistry*, **14**, 983 (1975).
13. W.G. Wright, *J. Chem. Soc., Perkin Trans. 1*, 1819 (1976).
14. V.K. Ahluwalia and N. Rani, *Indian J. Chem.*, **14B**, 594 (1976).
15. F. Bohlmann and W.-R. Abraham, *Phytochemistry*, **18**, 889 (1979).
16. C. Norbedo, G. Ferraro, and J.D. Coussio, *J. Nat. Prod.*, **45**, 635 (1982).
17. M. Aritomi and T. Kawasaki, *Chem. Pharm. Bull.*, **22**, 1800 (1974).
18. M. Maruyama, K. Hayasaka, S. Sasaki, S. Hosokawa, and H. Uchiyama, *Phytochemistry*, **13**, 286 (1974).
19. A. Sonn and W. Bülow, *Chem. Ber.*, **58**, 1691 (1925).
20. A.G. Doshi and B.J. Ghiya, *Curr. Sci.*, **55**, 502 (1986); *Chem. Abstr.*, **106**, 4608 (1987).
21. P. Klinke and H. Gibian, *Chem. Ber.*, **94**, 26 (1961).
22. E. Wollenweber and V.H. Dietz, *Phytochemistry*, **20**, 869 (1981).
23. W. Herz, S. Gibaja, S.V. Bhat, and A. Srinivasan, *Phytochemistry*, **11**, 2859 (1972).
24. H. Wagner, M.A. Iyengar, O. Seligmann, L. Höhrhammer, and W. Herz, *Phytochemistry*, **11**, 3086 (1972).
25. N. Fang, T.J. Mabry, and N. Le-Van, *Phytochemistry*, **25**, 235 (1986).
26. G.M. Laekeman, M. Claeys, P.C. Rwangabo, A.G. Herman, and A.J. Vlietinck, *Planta Med.*, **6**, 433 (1986).
27. N. Fang, M. Leidig, and T.J. Mabry, *Phytochemistry*, **20**, 869 (1981).

Received 26 August 1988