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UMUHENGERIN, A NEW ANTIMICROBIALLY ACTIVE FLAVONOID FROM *LANTANA TRIFOLIA*

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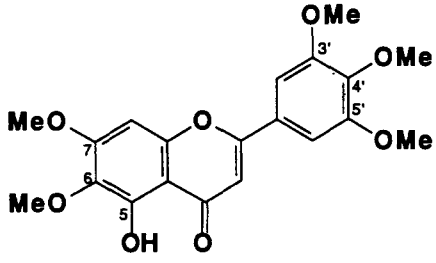
Some botanical species with a large use in folk medicine of Rwanda (1) have been screened for their antimicrobial (2,3), antifungal, and antiviral activities (4). *Lantana trifolia* L. (Verbenaceae) has been included in this study and has been selected for a more detailed investigation of its active principles. The genus *Lantana* is known to contain various terpenoid compounds (*Lantana camara* and *Lantana tiliaefolia*) (5–10), flavonoids and naphthoquinones (*Lantana archiranthifolia* (11), and cinnamylglycoside derivatives (*Lantana hybrida*) (12,13). To our knowledge no previous chemical or biological studies of *L. trifolia* have been reported.

We have isolated from the MeOH extract of the dried leaves a new, antimicrobially active, polymethoxylated flavone and have named it umuhengerin after the Rwandese name of the plant. The structure of the compound has been established as 5-hydroxy-6,7,3′,4′,5′-pentamethoxyflavone [**1**]. This substance has earlier been found to be present as a minor constituent in a sample of 5-hydroxy-6,7,3′,4′-tetramethoxyflavone but was never isolated from the mixture (14). It was tentatively identified by comparison of the ms and ^{1}H-nmr impurity peaks obtained for the mixture (15).



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Umuhengerin exhibited in vitro antibacterial and antifungal properties in concentrations up to 200 μg/ml against various pathogens including *Staphylococcus aureus, Salmonella typhimurium, Candida tropicalis, Aspergillus niger, Aspergillus fumigatus, Trichopyton mentagrophytes,* and *Microsporum canis*. It showed no antiviral activity in the concentrations tested. The results of the chemotherapeutic testing are presented in Table 1.

TABLE 1. Antibacterial and Antifungal Activities in vitro of Umuhengerin.

Microorganism	MIC[a] (μg/ml)
Staphylococcus aureus	20
Salmonella typhimurium	5
Candida tropicalis	20
Aspergillus niger	200
Aspergillus fumigatus	200
Trichophyton mentagrophytes	50
Microsporum canis	50
All other test microorganisms	>200

[a]MIC was defined as the lowest concentration of umuhengerin that inhibited macroscopic growth (16).

EXPERIMENTAL

PLANT MATERIAL.—Leaves of *L. trifolia* L. were collected in the Rwanda prefecture of Gisenyi in February 1983. A voucher specimen was deposited at the Laboratorium of Cuphamétra in Butare, Rwanda.

EXTRACTION AND ISOLATION OF UMUHENGERIN.—Dried leaves (1.582 kg) were first extracted with 13 liters of distilled H_2O, and after

rotary evaporation, the residue was extracted with 10 liters of 90% aqueous MeOH. The aqueous MeOH extract was concentrated and re-extracted with 0.5 liters of $CHCl_3$. After rotary evaporation, the $CHCl_3$ extract (23.04 g) was fractionated on a Si gel column (Si gel Merck 60, 230 mesh; 1.25 kg), which was successively eluted with 1 liter of petroleum ether 73°–94°, 1.5 liters of Et_2O, 1.5 liters of EtOAc, and 1 liter of MeOH. Fractions of 100 ml were collected, and a sample of every second fraction was analyzed on a Si gel tlc plate (25 mm). The tlc plate was developed with Et_2O-C_6H_6 (2:8), and detection was carried out by uv and by spraying with a 10% $SbCl_3$ in $CHCl_3$. Elution of the Si gel column with Et_2O resulted in 15 fractions, of which the last 10 were combined, concentrated by rotary evaporation, and resubjected to cc, as described above. This procedure yielded 60 mg (0.004% dry wt) of a yellowish compound. The compound was recrystallized from MeOH and showed a melting point of 193°–194°.

GENERAL EXPERIMENTAL PROCEDURES.—The mp was determined on a Büchi SMP-20 apparatus and was uncorrected. Uv spectra were obtained on a Beckmann Lambda 5 UV-instrument, and the ir spectrum was recorded on a Beckmann Acculab TM-4 instrument. ^{1}H- and ^{13}C-nmr spectra were obtained in $CDCl_3$ with a JEOL JNM-FX-200 operating at 199.5 MHz and 50 MHz, respectively. Chemical shifts are given in ppm values (δ) from TMS as internal standard. The mass spectrum was obtained with a Finnigan 4000 mass spectrometer connected to an Incos 2100 data system using direct probe introduction and electron impact.

IDENTIFICATION.—The compound exhibited the following properties: tlc on Si gel F_{254}, $CHCl_3$-HCOOH (100:2) showed under uv light of 366 nm a single dark-purple spot, R_f 0.55. No color change occurred with NH_3 vapor. With Naturstoffreagenz A (1% diphenyl-boric acid/ethanolamine complex in MeOH) spray reagent the color changed to green. Uv-visible spectra: λ max (MeOH) nm (log ε) 330 (4.43), 277 (4.34), NaOMe 293 (4.53), $AlCl_3$ 357 (4.46), 283 (4.35), $AlCl_3$/HCl 351 (4.47), 282 (4.39); ir ν max (KBr) cm^{-1} 3000–2800 (-OH), 1660 (-OMe); 1625 (α,β unsaturated CO), 1600 (C = C, aromatic); ^{1}H nmr (199.5 MHz, $CDCl_3$) δ 7.09 (2H, s, H-2′, H-6′), 6.61*, 6.56* (1H each, s, H-3, H-8), 3.94, 3.97, 3.99 (6H, 6H, 3H, s, OMe); ^{13}C nmr (50.1 MHz, $CDCl_3$) δ 182.6 (s, C-4), 163.8 (s, C-2), 158.9 (s, C-7), 153.7 (s, C-3′, C-5′), 153.2*, 153.1* (s, C-5, C-9), 141.6 (s, C-4′), 132.9 (s, C-6), 126.5, (s, C-1′), 106.3 (s, C-10), 105.4 (d, C-3), 103.9 (d, C-2′, C-6′), 90.7 (d, C-8), 61.0*, 60.8*, (s, OMe-4′, OMe-6) 56.5 (s, OMe-3′, OMe-5′) 56.4 (s, OMe-7); eims *m/z* $[M]^+$ 388 (100% rel. int.), $[M-H]^+$ 387 (17%), $[M-Me]^+$ 373 (74%), $[M-H-CO]^+$ 359 (17%), $[M-Me-CO]^+$ 345 (12%), $[359-OH]^+$ 342 (16%), 178 (16%), 153 (35%). An asterisk (*) means that the values may be interchanged.

CHEMOTHERAPEUTICAL EVALUATION.—Umuhengerin was chemotherapeutically tested by standard methods (16,17). The antibacterial and antifungal activities were determined in vitro with the dilution method against Gram-positive cocci including *Streptococcus pneumoniae, Micrococcus* sp., *Staphylococcus aureus, Streptococcus pyogenes,* and *Steptococcus viridans,* Gram-negative cocci including *Neisseria gonorrhoeae,* Gram-negative enteric bacilli such as *Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens,* and *Shigella flexneri,* the acid-fast bacillum *Mycobacterium fortuitum,* yeasts including *Candida albicans,* and *Candida tropicalis,* and fungi including *Aspergillus flavus, A. fumigatus, A. niger, Microsporum canis, Trichophyton mentagrophytes,* and *Trichophyton rubrum.*

The antiviral activity was determined in vitro with the tissue culture method using confluent VERO monolayers in microtiters plates against one DNA-virus (herpes simplex) and three RNA-viruses (coxsackie virus, poliovirus, and Semliki forest virus).

All bacteria and fungi used, except for *S. aureus* ATCC 25923, were from the collection of the Department of Microbiology, Faculty of Medicine, University of Antwerp. They were obtained as clinical isolates from patients diagnosed as having bacterial or fungal infections and typed. Poliomyelitis type 1 strain 1A/S2 was plaque-purified, and herpes simplex type 1 was isolated in the above-mentioned Department of Microbiology. Coxsackie B2 virus was obtained from NIH, Bethesda, Maryland, and Semliki forest L_{10} was supplied by Dr. C.J. Bradish, Microbiology Research Establishment, Porton Down, Salisbury, England.

Full details of experimental procedures are available upon request from the senior author.

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