Rottlerin Derivatives and Other Compounds from Mallotus philippinensis Fruits and Their Potential Antimycobacterial Activity

Rima Bharadwaj1, Ashish A. Chinchansure1, Roshan R. Kulkarni1, Manisha Arkile2, Dhiman Sarkar2, Swati P. Joshi1

1 Division of Organic Chemistry, CSIR-National Chemical Laboratory, Pune, India
2 Combi Chem-Bio Resource Centre, CSIR-National Chemical Laboratory, Pune, India

Abstract
The methanolic extract of the fruits of Mallotus philippinensis afforded 13 compounds, 7,11-diketo-lanost-3-ol (1, as acetate), lanosta-8-ene-3β-ol (2, as acetate), pregnenolone (3, as acetate), trans-chalcone (4), kamalachalcone E (5), oleanolic acid (6), gallic acid (7), kaempferol (8), myricetin (9), 1-(5,7-dihydroxy-2,2,6-trimethyl-2H-1-benzopyran-8-yl)-3-phenyl-2-propen-1-one (10), 4’-hydroxyisorottlerin (11), rottlerin (12), and shikimic acid (13). Compound 1 was isolated as a new natural product and its structure was elucidated by 1D and 2D nuclear magnetic resonance analyses including heteronuclear single quantum correlation, heteronuclear multiple-bond correlation, correlation spectroscopy, and nuclear Overhauser effect spectroscopy experiments. All of the isolated compounds were evaluated for their antimycobacterium activity against Mycobacterium tuberculosis H37Ra.

Key words
Mallotus philippinensis · Euphorbiaceae · antimycobacterium · rottlerins · lanostanol · flavonoids

Supporting information available online at http://www.thieme-connect.de/products

The genus Mallotus is a large genus of trees/shrubs distributed chiefly in the tropical and subtropical regions of the Old World with around 20 species in India [1]. The genus Mallotus is represented by five species in Maharashtra, and Mallotus philippinensis (Lam.) Muell.Arg. (Euphorbiaceae) is a branched tree distributed throughout the state [2]. A red dye called Kamala is secreted on the surface of the fruits and is used medicinally as well as commercially as a dye. It has a purgative property and is also used in the external applications for parasitic infections of the skin. It also has a lithotriptic property that dissolves or destroys stones in the kidneys and it is styptic (antihemorrhagic agent). Earlier work on the chemical analysis of different parts of this tree has revealed the occurrence of several triterpenes [3, 4], flavonoids [5–8], and dimeric chalcone derivatives [9, 10]. In our earlier work, from the acetone extract of whole uncrushed fruits of M. philippinensis, we isolated chalcones, including one new dimeric chalcone, kamalachalcone E, which was shown to have antifungal activity against the human pathogens Candida albicans, Candida glabrata, and Cryptococcus neoformans [11]. In the continuation of this work, we have examined the methanol extract of the crushed fruits of M. philippinensis to isolate 13 compounds (1–13) and evaluated them for antimycobacterium activity against Mycobacterium tuberculosis H37Ra.

Compound 1 was isolated as an acetate. Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) showed pseudomolecular peaks at 501.02 [M + 1] and 539.01 [M + K], corresponding to the molecular formula C32H52O4 with seven degrees of freedom. The 1H NMR (Fig. S1, Supporting Information) spectrum showed five singlet methyls at δH 0.72, 0.84, 0.92, 1.21, and 1.30, one three proton doublet methyl at δH 0.85 (3 H, d, 6.0 Hz), one six proton doublet methyl at 0.87 (6 H, d, 6.7 Hz), and seven methines at δH 1.35, (m), 1.53 (s, 6.9 Hz), 1.60 (m), 1.26 (m), 2.22 (d, 12.9 Hz), 2.67 (d, 12.7 Hz), and 4.51 (dd, 7.6, 5.6 Hz). The 13C NMR (Fig. S2, Supporting Information) and distortionless enhancement by polarization transfer (DEPT, Fig. S3, Supporting Information) spectra showed the presence of eight methyls, seven methylenes, seven methines, and six quaternary carbons, including two carbonyl groups at δC 209.0 and 209.6. This data suggests that compound 1 was a tetracyclic triterpenoid belonging to the class lanostanol. The complete structure was elucidated by 2D NMR. A proton at δH 2.59 (H-12) showed an HMBC correlation with the carbonyl carbon at δC 209.6 (C-11), while a proton at δH 1.26 (H-5) showed an HMBC correlation with the carbonyl carbon at δC 209.0 (C-7). The key HMBC correlations are shown in Fig. S4, Supporting Information. NOESY correlations were observed between H3-18 (δH 0.72) and H-8 (δH 2.67), between H3-19 (δH 1.30) and H-8 (δH 2.67), H3-29 (δH 0.91), and between H3-21 (δH 0.85) and H-9 (δH 2.22), which led to the assignment of the relative stereochemistry (Fig. S4, Supporting Information). Thus, 1 was identified as 7,11-diketo-lanost-3β-ol. It has been prepared synthetically and reported earlier [12]. To the best of our knowledge, this is the first report of the isolation of compound 1 as a new natural product.

Compounds 2–13 were identified as lanosta-8-ene-3β-ol (2, as acetate) [13], pregnenolone (3, as acetate) [14], trans-chalcone (4), kamalachalcone E (5) [11], oleanolic acid (6) [15], gallic acid (7) [16], kaempferol (8) [17], myricetin (9) [18], 3.3.2 1-(5,7-dihydroxy-2,2,6-trimethyl-2H-1-benzopyran-8-yl)-3-phenyl-2-propen-1-one (10) [11], 4’-hydroxyisorottlerin (11) [10], rottlerin (12) [11], and shikimic acid (13) [19] (Fig. 1) by comparison with the literature NMR data and were confirmed by the LC-ESI-MS. An attempt has been made to define the configuration of compound 11 by comparing it with structurally similar flavanone derivatives. Compound 11 was determined to have a negative specific rotation, [α]D25°C = –12.11 (c 0.6, chloroform). Literature analysis suggests that the negative specific rotation was consistent with the a orientation of the group at position 2 [20–22]. This allowed for the determination of the a orientation of the group at position 2 in compound 11. Compounds 1–13 were evaluated for their antimycobacterium activity against M. tuberculosis H37Ra. Compounds 11 and 12 exhibited antimycobacterium activity with IC50 values of 0.89 ± 0.33 µg/mL (MIC 2.06 ± 0.41 µg/mL) and 7.59 ± 0.42 µg/mL (MIC 11.56 ± 0.35 µg/mL), respectively, as compared to the positive control rifampicin.

Materials and Methods

1H and 13C NMR spectra were recorded on a Bruker Avance III Ultra Shield NMR (1H operating frequency: 400 MHz) instrument. LC-ESI-MS was recorded with the Waters Acquity LC-MS instru-
ment. Optical rotations were measured with a JASCO P-1020 polarimeter. The Spectramax plus 384 plate reader (Molecular Devices, Inc.) was used. All the solvents used were distilled prior to use. Column chromatography (CC) was performed with silica gel purchased from Thomas Baker Ltd. Preparative thin-layer chromatography (TLC) was carried out using TLC plates supplied by Merck Ltd. The *M. tuberculosis* H37Ra (ATCC 25177) strain was obtained from MTCC, Chandigarh, India. Rifampicin (97.0%), XTT sodium salt, and menadione were purchased from Sigma-Aldrich.

Plant material
Fruits of *M. philippinensis* were collected from Bhimashankar Forest area, Pune District in March 2012. A herbarium voucher is deposited at Botanical Survey of India, Western Circle, Pune (No. PCOPAVMA2).

Extraction and isolation
Whole fruits obtained after acetone extraction were dried under airflow and pulverized. The pulverized fruit powder, 1.2 kg, was extracted with methanol by a cold maceration technique (3 L × 3 × 14 h) at room temperature. After solvent evaporation at a reduced pressure, a red-brown extract (31.0 g, 2.58%, based on dry weight) was yielded. The methanol extract, 30 g, was separated by repeated CC on silica gel with different elution systems. The final purification of the compounds was achieved by preparative TLC. The detailed extraction and isolation of the compounds 1–13 as well as LC-ESI-MS, UV, IR, and NMR data of the compounds are available as Supporting Information.

Antimycobacterium bioassay using the XTT reduction menadione assay
*M. tuberculosis* H37Ra cells (ATCC 25177) were grown to the logarithmic phase (O.D.595 ~ 1.0) in M. phlei medium. Compounds 1–13 were dissolved in dimethyl sulfoxide and stored in aliquots at −20°C. A freshly prepared stock solution (1.25 mM) of XTT sodium salt in sterile 1 x PBS and 6 mM menadione in DMSO was used. Compounds were screened for their inhibitory effect on *M. tuberculosis* by following the XTT reduction menadione assay (XRMA) protocol [23,24]. Briefly, in all wells of the assay plate, 200 µM XTT were added and incubated at 37°C for 20 min. Menadione, 60 µM, was added and incubated at 37°C for 40 min. The
optical density was recorded on a microplate reader at 470 nm (filter) against a blank prepared from cell-free wells. Absorbance given by the cells treated with the vehicle alone was taken as 100% of cell growth. All the experiments were performed in triplicate and the quantitative values are expressed as the average ± standard deviation, and the IC\textsubscript{50} values were calculated from their dose-response curves (Figs. S5 and S6, Supporting Information). Compounds 11 and 12 exhibited promising inhibitory activity with IC\textsubscript{50} values of 0.89 ± 0.33 µg/mL (MIC 2.06 ± 0.41 µg/mL) and 7.59 ± 0.42 µg/mL (MIC 11.56 ± 0.35 µg/mL), respectively, as compared to that of positive control rifampicin with an IC\textsubscript{50} value of 0.0019 ± 0.0003 µg/mL (MIC 0.02 ± 0.31 µg/mL).

Supporting information 1\textsuperscript{H}, 13\textsuperscript{C}, DEPT, and 2D correlations of compound 1, dose-response curves for compounds 11 and 12 against M. tuberculosis H37R\textalpha{}, and the extraction, isolation, NMR, and other characterization data of compounds 1–13 are available as Supporting Information.

Acknowledgments

We are thankful to Dr. Sagar Pandit, Division of Biochemical Sciences, CSIR-NCL, Pune for identifying the plant material. We are grateful to the University Grants Commission, New Delhi, India, for providing financial support.

Conflict of Interest

There is no conflict of interest among all authors.

References


received May 14, 2015
revised June 30, 2015
accepted June 30, 2015

Bibliography

DOI http://dx.doi.org/10.1055/s-0035-1557793
© Georg Thieme Verlag KG Stuttgart · New York · ISSN 2199-157X

Correspondence

Dr. Swati P. Joshi
Division of Organic Chemistry
CSIR-National Chemical Laboratory
Dr. Homi Bhabha Road
Pune-411008, Maharashtra
India
Phone: +91 20 2590 2327
Fax: +91 20 2590 2627
swatijoshi@gmail.com

Bharadwaj R et al. Rottlerin Derivatives and ... Planta Med Lett 2015; 2: e28–e30