

A New Oxetane of *Citrus Microcarpa*T.L. Yang¹, H.C. Yeh², H.T. Li³, S.L. Liu⁴, and C.Y. Chen^{2*}¹Department of Clinical Microbiology Laboratory, Yuan's General Hospital, Kaohsiung, Taiwan.²School of Medical and Health Sciences, Fooyin University, 83102, Kaohsiung, Taiwan.***Correspondence:**

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ABSTRACT

A new oxetane, 3-methoxy-2-methyloxetan-3-amine (**1**) was isolated from the seeds of *Citrus microcarpa* Bunge (*Rutaceae*). The structure of the new oxetane was elucidated by chemical and physical evidence.

Keywords

Citrus microcarpa, *Rutaceae*, Oxetane.

The genus *Citrus* is believed to have originated from Southeast Asia. Nowadays, it comprises hundreds of varieties and hybrids as a result of natural or artificial crossbreeding. Conventional citrus fruits (e.g., oranges, lemons, grapefruits, limes and mandarins) have been widely studied and produced commercially [1]. Recently, exotic citrus fruits have attracted attention worldwide, due to their unique sensory attributes and health benefits [2]. *Citrus microcarpa* Bunge plant, locally known as kalamansi, is a member of Family *Rutaceae* that is native and widely cultivated in the Philippines. The present study was undertaken to study the hepatoprotective activity of fruit peel from kalamansi [3]. Earlier investigations on the chemical constituents of seeds of *C. microcarpa* with 12 compounds, including four long-chain hydrocarbones, palmitic acid, stearic acid, linoleic acid and methyl palmitate, four benzenoids, vanillic acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde and methylparaben, one terpenoid, squalene and three steroids, β -sitosterol, β -sitostenone, and stigmastenone [4]. In the course of screening for biologically and chemically novel agents from Formosan plants in the family *Rutaceae* [4-6], *C. microcarpa* was chosen for further phytochemical investigation. In this paper, we report the isolation and structural elucidation of this new oxetane.

3-Methoxy-2-methyloxetan-3-amine (**1**), obtained as a yellow oil, established by the molecular formula $C_5H_{11}O_2N$ by HR-EIMS at

m/z $[M + Na]^+$ 140.0689 (calcd for $C_5H_{11}O_2NNa$, 140.0687). Two IR bands at ν_{max} 3300 and 1017 cm^{-1} suggested that primary amine group and a cyclic ether group might be present. The ¹H NMR spectrum of **1** showed one methine proton at δ 3.97 (1H, m) for H-2, one methylene protons at δ 3.20 (1H, dd, $J = 9.6, 7.8$)/3.37 (1H, dd, $J = 9.6, 3.0$) for H-4 and two methyl protons at δ 1.14 (3H, d, $J = 6.0, 2-CH_3$) and 3.39 (3H, s, 3-OCH₃). ¹³C NMR and DEPT experiments on **1** showed 5 resonance lines consisting of two methyls, one methylene, one methines and one quaternary carbon. The mass, UV, IR and ¹H NMR data suggested that **1** is a type of oxetane and that the position of methyl, methoxyl and amine groups should be located on the skeleton. The sequential correlations of the NOESY spectrum were successfully established as shown in Figure 1. Thus, the structure of this compound was named to be 3-methoxy-2- methyloxetan-3-amine (**1**), which was further confirmed by HMBC experiments (Table 1).

Table 1: NMR data of **1** in CDCl₃ (δ in ppm, J in Hz, 600 MHz for ¹HNMR, and 150 MHz for ¹³C NMR).

Position	δ_c	δ_H	mult., J (Hz)	HMBC (¹ H \rightarrow ¹³ C)
2	66.4	3.97	m	C-3, 2-Me
3	77.0	-	-	-
4	78.3	3.20 3.37	dd, 9.6, 7.8 dd, 9.6, 3.0	C-3 C-3
2-Me	18.7	1.14	d, 6.0	C-2
3-OMe	59.0	3.39	s	C-3

Experimental

General

UV spectra were obtained in CH₃CN, IR spectra were measured on a Hitachi 260-30 spectrophotometer. ¹H NMR (600 MHz), ¹³C NMR (150 MHz), HETCOR, HMBC, COSY, NOESY, and DEPT spectra were obtained on a Varian (Unity Plus) NMR spectrometer. Low-resolution ESI-MS spectra were obtained on an API 3000 (Applied Biosystems) and high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer. Silica gel 60 (Merck, 70~230 mesh, 230~400 mesh) was used for column chromatography. Precoated Silica gel plates (Merck, Kieselgel 60 F-254), 0.20 mm and 0.50 mm, were used for analytical TLC and preparative TLC, respectively, visualized with 50% H₂SO₄.

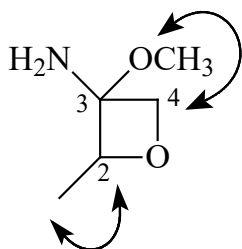


Figure 1: NOESY correlations of **1**.

Plant Material

The specimen of *Citrus microcarpa* Bunge was collected from Kaohsiung City, Taiwan in January, 2017. A voucher specimen was identified by Professor Fu-Yuan Lu (Department of Forestry and Natural Resources College of Agriculture, National Chiayi University) and was deposited in the School of Medical and Health Sciences, Fooyin University, Kaohsiung, Taiwan.

Extraction and Isolation

The seeds (33.5 g) of *C. microcarpa* were air dried and extracted repeatedly with MeOH (300 mL × 5) at room temperature. The combined MeOH extracts (5.8 g) were then evaporated and further

separated into 5 fractions by column chromatography on silica gel (1.6 kg, 70-230 mesh) with gradients of *n*-hexane/CH₂Cl₂/MeOH. Part of fraction 4 (0.8 g) was subjected to silica gel chromatography by eluting with CH₂Cl₂-MeOH (80:1), enriched with MeOH, to furnish three fractions (4-1-4-3). Fraction 4-2 (0.1 g) was further purified on a silica gel column using CH₂Cl₂/MeOH mixtures to obtain 3-methoxy-2-methyloxetan-3-amine (**1**) (3 mg).

3-Methoxy-2-methyloxetan-3-amine (1). Yellow oil. $[\alpha]_D^{25} +13.4$ (*c* 0.55, CHCl₃). UV λ_{max} (MeCN, log ϵ): 210 (3.11) nm. IR (ν_{max} , cm⁻¹): 3300 (NH₂), 1017 (ether). ESI-MS *m/z* 140 [M+Na]⁺; HR-ESI-MS *m/z* 140.0689 [M+Na]⁺ (calcd for C₅H₁₁O₂NNa, 140.0687). ¹H and ¹³C NMR data, see Table 1.

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