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A NEW ACYLATED KAEMPFEROL DERIVATIVE FROM *LILIUM CANDIDUM* L.

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Key Word Index—*Lilium candidum*; Liliaceae; tetrahydroxymethylsuccinoylflavone; lilaline; biosynthesis.

Abstract—3,5,7,4′-Tetrahydroxy-8-(3″-methylsuccinoyl)-flavone, a likely precursor of the alkaloid lilaline has been isolated from the aerial part of *Lilium candidum*.

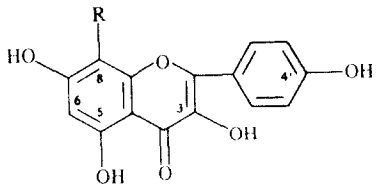
INTRODUCTION

Recently we described the isolation and structure elucidation of the unusual flavone alkaloid lilaline [1] from the aerial parts of *Lilium candidum* L. Other constituents of the plant include pyrroline–pyrrolidine alkaloids [2, 3] kaempferol [4] and some organic acids [5]. In a further study of this plant we have now obtained a new acylated flavone derivative 3,5,7,4′-tetrahydroxy-8-(3″-methylsuccinoyl)-flavone **2** which is an obvious biogenetic precursor of lilaline **3**.

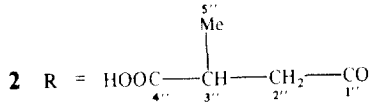
RESULTS AND DISCUSSION

Extraction of dried flowers of *Lilium* with aqueous ethanol followed by chromatography of the extract over silica gel afforded a crystalline acid, $C_{20}H_{16}O_9$, whose UV spectrum (λ_{max} 371, 322, 242 and 249 nm) is consistent [6] with the presence of a C-acylated flavonol nucleus. Its IR spectrum (3410, 3100–2000, 1715, 1650 and 840 cm^{-1}) supported this proposal and confirmed the presence of a carboxyl and carbonyl functions. A fragment in the MS at *m/z* 286 suggested that the flavonoid moiety is kaempferol [4]. Its C-substitution was verified using ^{1}H NMR spectroscopy, where in addition to the four protons of the C ring, a singlet at δ6.21 (H-6 or H-8) was observed. ^{1}H NMR spectrum further revealed the presence of a Me-CH-CH_2-fragment in the molecule. Considering the MS and IR data the presence of additional carboxyl and carbonyl group becomes evident. ^{13}C NMR spectroscopy supported this assumption (carbonyl at δ203.1, carboxyl δ180.0) and was used to identify the site of substitution of this fragment by a carboxylic group. The presence of long-range proton–carbon coupling [7] between methyl protons and carboxyl group places it unambiguously at the methine carbon (C-3″), adjacent to the methylene carbon (C-2″) bound to a carbonyl group. Also the site of substitution of kaempferol can be elucidated using the ^{13}C NMR spectrum. In comparison with corresponding carbons of kaempferol [8] C-7, C-8 and C-8a carbons of **2** displayed an upfield shift up to about 8 ppm, while on the opposite C4a, C-5 and C-6 were shifted by less than 2 ppm. These changes confirmed that the kaempferol is substituted at C-8. The position of ^{13}C NMR signals of succinoyl moiety of **2** is in agreement with the position of respective carbons observed in 2-methyl-4-oxo-4-(2-hydroxy-6-methoxyphenyl)-butanoic acid [9]. Thus the new compound is 3,5,7,4,′-tetrahydroxy-8-(3″-methylsuccinoyl)-flavone **2**.

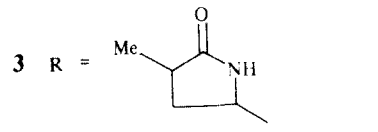
The proposed biosynthetic route to lilaline **3** includes C-acylation of kaempferol with 2-methylsuccinic acid to give the acylated derivative **2** followed by the introduction of the nitrogen atom. The isolation of **2** provides strong support for this pathway. Kaempferol and methylsuccinic acid are already known constituents of *L. candidum* [4, 5].



1 R = H



2 R = HOOC—CH(Me)—CH_2—CO



3 R =

EXPERIMENTAL

The plant material, *Lilium candidum* L., was collected in South Slovakia and the herbarium specimen is deposited in the Department of Pharmacognosy and Botanics of the Pharmaceutical Faculty, Comenius University, in Bratislava. Mps: uncorr.

Extraction and isolation of 8-(3-methylsuccinoyl)-kaempferol. Dry flowers (1500 g) were macerated several times at room temp. with 96 and 70% EtOH. The extract was filtered and evapd. The residue (699 g) was dissolved in 5% HCl and substances present in acidic soln were extracted successively with petrol, Et_2O and $CHCl_3$. Chromatography of the crude ether fraction (30 g) after removal of the solvent over silica gel using benzene–acetone gave 440 fractions. Rechromatography of fractions 64–101 over silica gel using benzene–acetone afforded 3,5,7,4′-tetrahydroxy-8-(3″-methylsuccinoyl)-flavone (27.5 mg). Mp 221–222°.

UV λ_{max}^{MeOH} nm: 242, 249, 322, 371. IR: 840, 1615, 1650, 1715, 2000–3100, 3410 cm^{-1}. Mass: M^+ 400 $C_{20}H_{16}O_9$ *m/z* 382, 367, 355 $C_{19}H_{15}O_7$, 338 $C_{19}H_{14}O_6$, 337, 323, 313, 286, 258, 257, 229, 213. ^1H NMR (300, 13 MHz, CD_3OD, TMS has been used as int. standard): δ8.01 (2H, *d*, $J_{2',3'}$ = 8,9 Hz, H-2′, H-6′), 6.92 (2H, *d*, H-3′, H-5′), 6.21 (1H, *s*, H-6), 3.68 (1H, *dd*, $J_{2a'',2b''}$ = 18.7 Hz, $J_{2a'',3''}$ = 9.6 Hz, H-2a″), 3.37 (1H, *dd*, $J_{2b'',3''}$ = 4.5 Hz, H-2b″), 3.07 (1H, *dqd*, $J_{3'',5''}$ = 7.3 Hz, H-3″), 1.27 (3H, *d*, H-5″). ^{13}C NMR δ203.1 (C-1″, *s*), 180.0 (C-4″), 177.2 (C-4), 171.6 (C-7), 167.3 (C-8a), 160.9 (C-4′), 158.9 (C-5), 150.2 (C-2), 138.1 (C-3), 131.5 (C-2′, C-6′), 116.4 (C-3′, C-5′), 122.8 (C-1′), 105.2 (C-8), 104.5 (can be exchanged) (C-4a), 100.3 (C-6), 48.8 (solvent Me_2O in MeOH, overlapped by the solvent) (C-2″), 36.2 (C-3″), 17.9 (C-5″).

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A CYCLOPEPTIDE ALKALOID FROM THE BARK OF *ZIZYPHUS RUGOSA*

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Key Word Index—*Zizyphus rugosa*; Rhamnaceae; bark; alkaloids; rugosanine-A; *N*-formylcyclopeptide alkaloid; uncharacterised norcorypalline.

Abstract—Rugosanine-A has been isolated from the stem bark of *Zizyphus rugosa*. The structure was deduced by spectroscopic methods and chemical degradation. It is a 13-membered *N*-formylcyclopeptide alkaloid and provides the third example of such a naturally occurring *N*-formylcyclopeptide alkaloid.

INTRODUCTION

Zizyphus rugosa Lam. (Family: Rhamnaceae) is a large shrub distributed throughout India. The bark of the plant is commonly used in the Indian medicine for the treatment of diarrhoea while the flowers, together with leaves, are used in menorrhagia [1]. In continuation of our search for the peptide alkaloids from the bark of *Z. rugosa* [2], we now report here the isolation and characterisation of a new 13-membered *N*-formyl cyclopeptide alkaloid, rugosanine-A (**1**) together with an uncharacter-

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