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New Butyl Shikimate Acetal Derivative from Aerial Parts of Acalypha wilkesiana Müll. Mosaica. extracts

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Abstract

The aerial parts of *Acalypha wilkesiana* gave undescribed potassium 2-butoxyacetimidate (1) and butyl 3(*S*),4(*S*),5(*R*) -4-oxy-3- epishikimic acid carboxylate acetal [(1*S*,8*S*, 9*R*) -8,9-dihydroxy-3-propyl-2,4-dioxabicyclo [4.2.2] dec-6-en-5-one] (2), and shikimic acid, as well as nineteen known glycosylated phenylpropanoids, benzoic acid derivatives and flavonoids. The structures of the isolated compounds were elucidated based on 1D, 2D NMR, and ESI MS.

1. Introduction

Acalypha wilkesiana (A. wilkesiana), family Euphorbiaceae, is a traditional medicinal plant, distributed in Africa and Asia (Wijaya *et al.* 2011). Extracts of the leaves of *Acalypha wilkesiana* (Macrophylla) have been shown to possess antibacterial and antifungal activity and were found to be reasonably useful in the treatment of eczema (Sherifat *et al.*2022). It is used in anticancer treatment for lung, breast, and brain cancers (Lim et al. 2013). Methanol leaf extract of the plant conferred protection to the kidney of rats against chronic cyanide toxicity by ameliorating renal indices and up-regulating antioxidant response (Kadiri, and Ossai 2023). Previous research on phytochemical screening of *A. wilkesiana* leaves reported the presence of saponins, tannins, anthraquinones, and glycosides. In addition to separating shikimic acid, some flavonoids and phenolic compounds such as quercetin 3-*O*-rutinoside, naringenin, kaempferol, kaempferol-3-*O*-α-L-rhamnoside, apigenin, artemetin, luteolin, vitexicarpin, (−) epicatechin, (+) -catechin, tannic acid, 3,6- (*R*) - hexahydroxydiphenoyl (α/β)- $^{1}C_{4}$-glucopyranose, punicalin, corilagin, geraniin, brevifolin carboxylic acid, methyl brevifolin carboxylate, gallic acid, methyl gallate, ellagic acid and monomethoxy ellagic acid (El-raey et al. 2016). The pharmaceutical industry used shikimic acid, which was derived from medicinal plants, primarily Chinese star anise (Illicium verum) (Ramazani et al. 2021).

This study focuses on isolating the flavonoids and phenolic compounds in *A. wilkesiana* extracts, that act as antioxidants.

2. Results and Discussion

Nineteen compounds were isolated from n-butanol extract (BE) and three from ethyl acetate extract (EA). Some of the compounds were isolated as single compounds, while others were obtained as combinations of two or three compounds. Along with well-known compounds (**3–22**), two novel compounds (**1** and **2**) were isolated for the first time. A few ethyl esters are among the detected components (compounds **8**, **9**, **11**, **12**, and **13**), however Venditti et al. (2020) believe that they are more likely artefacts.

Compounds isolated from (EA)

Compound (1) (Potassium butoxyacetimidate) (new compound):

Compound **1** was eluted from a polyamide CC of ethyl acetate extract by water. Thus it was expected to be a polar or ionic compound, which was also in agreement with its solubility in water and in DMSO. The positive ionization ESI MS gave a molecular ion peak at m/z 169 in agreement with $C_6H_{12}NO_2K$ indicating a potassium salt. No alkali was used in extraction, which eliminates any possibility that **1** is an artefact.

^{1}H NMR spectrum of compound **(1)** showed a triplet of a primary methyl group with coupling constant of 7.2 Hz at δ 0.87 coupled with a methylene group multiplet at δ 1.36–1.22, which coupled with a methylene group quintet with coupling constant of 7.3 Hz at δ 1.45, which in turn coupled with an oxygenated methylene triplet with coupling constant of 6.9 Hz at δ 3.37, as indicated by cross peaks in the H, H COSY spectrum. The APT ^{13}C NMR, and HSQC spectra all supported the presence of a butyloxyl group. The four carbon signals in the butyloxyl group, as well as an oxygenated methylene group (δ_c 71.60 and δ_H 3.50) and a carboxylate derivative carbon at δc 172.52, were viewed in the APT ^{13}C NMR spectrum, which indicated its identity as potassium butoxyacetimidate. The cross-peaks due to $^2J_{C1,H2}$; $^3J_{C2,>H3}$; $^3J_{C3,H2}$; $^3J_{C5,H3}$; $^3J_{C4,H6}$ and $^2J_{C5,H6}$ in the HMBC spectrum have validated the structure. Additionally, a cross peak between H-2 and H-3 was seen in the NOESY spectrum, confirming their proximity. Further confirmation of the structure of compound **1** was achieved from comparing the ^{1}H and ^{13}C NMR spectral data of the acetimidate moiety with those of ethoxyacetic acid and its ethyl ester (Oláh et al. 2018). Figure 1 depicts the compound (1)'s structure, while Fig S1a–f displayed its spectra.

Figure 1. Structure of compound **(1)** (potassium butoxyacetimidate)

Compound (3) [Shikimic acid]:

An olefinic proton broad singlet was visible in the compound **(3)**'s ^{1}H NMR spectra (Singh et al. 2020) at δ_H 6.43 ppm, crossing a peak in H, H COSY, with a signal of -CH-O at δ_H 4.16 ppm. It has a cross peak in H, H COSY and a signal of another -CH-O at δ_H 3.45 ppm. It, in turn, has a cross peak in H, H COSY, with a signal at δ_H 3.77 ppm, denoting the presence of a third -CH-O, which has two cross peaks in H, H COSY, two signals of CH_2, and a geminal coupling of 18 Hz at δ_H 1.98, 2.47 ppm.

Seven carbon signals including a carboxylic carbonyl at δ_C 171.02, two olefinic carbons at δ_C 132.92, 134.29, three oxygenated carbons at δ_C 65.91, 67.36, 72.06, and one nonoxygenated sp^3 carbon at δ_C 31.87 ppm were detected in the ^{13}C NMR spectrum. The HSQC spectrum gave the $^1J_{H,C}$ correlations. Shikimic acid and these data (Figs S3 and S4a-g) were well-concordant (Singh et al. 2020, Caprioli et al. 2016, Venditti et al. 2017). Its positive ESI MS (Fig S4h) produced a quasi-molecular ion peak at m/z 192 due to $[M + NH_4]^+$in accordance with the molecular formula $C_7H_{10}O_5$.

Compound (4) (luteolin-4'-O-rutinoside)

It was predicted to be luteolin-4'-O-rutinoside based on its chromatographic behavior and ^{1}H NMR (Albayrak et al. 2021). Fig S5 depicted the structure, while Fig S6 displayed the ^{1}H NMR spectrum.

Compounds isolated from (BE)

Compound (2):

The signals of shikimic acid (**3**) were visible in the ^{1}H NMR spectrum of compound (**2**) (Fig S2a), along with some shifts in δ_H values [6.67, *br s*, H-2; 4.57, *dd* (3.3,7.5), H-3; 3.92, *t* (6.7), H-4; 3.65, *m*, H-5; 2.11, *dd* (7.5,17.0), H-6$_{ax}$; 2.41, *dd* (3.8,17.0), H-6$_{eq}$], adding to the four signals that create the butyl acetal spin system, $CH_3CH_2CH_2C(-O)H-O$ [4.94, *t* (4.5), H-1'; 1.51, *m*, H-2'; 1.33, *m*, H-3'; 0.86, *t* (7.3), H-4']. The H, H COSY spectra (Figs S2c-e) provided cross peaks between H-2, H-3, H-3, H-4, H-4, H-5, H-5, H-6$_{ax}$, H-6$_{eq}$, and between H-1', H2'; H-2', H-3'; H-3', H-4', which corroborated the two spin systems of both the shikimate and the butyl acetal moieties. ^{13}C NMR (Fig S2b) and the HSQC spectrum (Fig S2g) indicated eleven carbon signals of 11 carbon atoms. One carbonyl carbon signal was found at δ_c 168.00, two olefinic carbon signals were found at δ_c 128.92 and 133.18, three oxygenated sp^{3}carbon signals were found at δ_c 72.79, 77.66, 67.48, and an acetal carbon signal was found at δ_c 103.80 ppm. Along with other cross peaks with C-2, C-3, C-5, and C-6, the HMBC spectrum (Fig S2f) revealed a cross peak between H-4 and C-1', showing the acetal link to C-4, in addition to a weak cross peak could be seen by expansion between H-1' and C-7. Figs S2a,f showed additional significant HMBC connections. According to Chen et al. (2011), the down-field shift of the δ_H and δ_c values of atoms 1' from 4.08, 65.8 in butyl shikimate to 4.95, 103.8 in compound (**2**) gave more support to the link of C1' to the carboxylate group. In accordance with $C_{11}H_{16}O_5$, the negative ionisation ESI MS revealed M-H at m/z 227, indicating its molecular ion 228 (Fig S2h). Based on coupling constant, *J* values, as well as comparing δ_c values with the corresponding in butylshikimate, the stereochemistry around C-3, C-4, and C-5 was determined (Chen et al. 2011). H-3's axial position was indicated by the large value of $J_{3,4}$ (7.5 Hz), and H-4 and H-5's axial positions were indicated by the large value of $J_{4,5}$ (6.7 Hz). The δ_c of atom 3 underwent significant shifts (from δ_c 67.4 in butyl shikimate to 72.8 in compound 2, or + 5.4 ppm), and δ_c of atom 4 (moving up by 5.0 ppm from δ_c 72.7 in butyl shikimate to δ_c 77.7 in compound (**2**), although C-5 only shifted slightly (from δ_c 68.5 in butyl shikimate to δ_c 67.5 in compound (**2**), a difference of 1.0 ppm), suggesting the same stereochemistry at C-5, and C-3, C-4, and C-5 all have a *trans*, *trans* configuration. The stereochemistry surrounding the acetal carbon (C1') could not be identified because of the several conformations that have been proposed for cyclooctane, with the boat-chair being the prevalent conformation (Meiboom et al. 1977). As H-4 should have an equatorial orientation on the cyclohexene ring, which is predicted to have a stiff bicyclic shape and bear a pseudo-boat-like conformation (S6d). As a result, compound (2) was recognized as a novel acylacetal, Butyl 3(*S*),4(*S*),5(*R*) -4-oxy-3-epishikimic acid carboxylate acetal [(1*S*,8*S*,9*R*) -8,9-dihydroxy-3-propyl-2,4-dioxabicyclo [4.2.2] dec-6-en-5-one], Fig. 2. To the best of our knowledge, this is the first naturally occurring acylacetal to be reported, and no acylacetals have ever been synthesized.

Figure 2. Structure of compound **(2)** Butyl 3(*S*),4(*S*),5(*R*) -4-oxy-3-epishikimic acid carboxylate acetal

Compounds (**5–7**) were identified as 3-*O*-*E*-feruloylquinic acid (**5**) (Holub et al. 2019), 3-*O*-*E*-caffeoylmethylquinate (**6**), and gallic acid 4-*O*-β-glucopyranoside (**7**) (Bai et al. 2021) based on their chromatographic behavior and ^{1}H NMR (Figs S7, S8a-b). Comparing the two molecules (**8**) and (**9**) to structurally related compounds (Huang et al. 2017) (Figs S9 and S10a-b), it was determined that they were 5-*E*-caffeoylquinic acid methyl ester (chlorogenic acid methyl ester, **8**) and 5-*E*-caffeoylquinic acid ethyl ester (chlorogenic acid ethyl ester, **9**) respectively.

Compounds **(10–13)** were identified as protocatecheuic acid (**10**) (Kakkar 2014), methyl gallate (**11**), gallic acid 4-*O*-methyl ether (**12**) and ethyl gallate (**13**) (Yang et al. 2014) (Figs S11 and S12a-b). Compounds **(14–16)** were identified as kaempferol-, quercetin-, myrecetin 3-*O*-*α*-rhamnopyranosyl-(1‴→2″)-β-glucopyranoside (**14–16**) (Halabalaki et al. 2011; Mahmoud et al. 2001) (Figs S13 and S14a-b). Compounds (**17 & 18)** were identified as kaempferol 3-*O*-α-L-$^{1}C_{4}$-rhamnopyranosyl-(1‴→6″)-β-D-*O*-$^{4}C_{1}$-glucopyranoside 3″-sulphate (kaempferol 3-*O*-β-**D**-rutinoside 3″-sulphate, **17**) and kaempferol 3-*O*-α-**L**-$^{1}C_{4}$-rhamnopyranosyl-(1‴→6″)-β-**D**-*O*-$^{4}C_{1}$-glucopyranoside 4″-sulphate (kaempferol 3-*O*-β-D-rutinoside 4″-sulphate, **18**), were shown in Figs S15 and S16a-b (Agrawal 1989; Mabry et al. 1970). Compound **(19)** was confirmed as kaempferol 3-*O*-β-robinobioside 4″-gallate (Grayer and Harborne 1994; Khan et al. 2017; Mahmoud et al. 2001; Moustafa et al. 2009; Yedidia et al. 2019) (Figs S17 and S18a-b). Compounds **(20–22)** were identified as kaempferol 3-*O*-β-D-robinobioside (**20**), quercetin 3-*O*-β-D-robinobioside (**21**) and kaempferol 3-*O*-β-D-rutinoside (**22**) (Grayer and Harborne 1994; Khan et al. 2017) (Figs S19 and S20a-b).

3. Experimental

General experimental procedures

All details are provided in the supplementary material. Plant material, materials and methods, equipment, extraction and isolation, identification of compounds, and spectral data of all compounds were provided in supporting information.

4. Conclusions

For the first time, two novel compounds from *A. wilkesiana* were isolated and identified as natural products: Butyl 3(*S*),4(*S*),5(*R*) -4-oxy-3-epishikimic acid carboxylate acetal [(1*S*,8*S*,9*R*)-8,9-dihydroxy-3-propyl-2,4-dioxabicyclo[4.2.2]dec-6-en-5-one], and potassium 2-butoxyacetimidate. Along with shikimic acid, there are nineteen other flavonoids and polyphenols that are well-known.

Declarations

Conflict of Interest

The authors declare no conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Competing interests

(The authors did not receive support from any organization for the submitted work)

Authors' contributions

Amal M. Youssef Moustafa supervised on experimental part of the research article, wrote and revised of the article, and elucidate structures of isolated compounds, Maha M. A. El-Damrany did the experimental part, Mamdouh Abdel-Mogib helped in the elucidation of the structures of the two new compounds. Magdy M. Youssef supervised on the research article.

Funding

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Availability of data and materials

(The phenolic compounds that were identified may be used as cancer treatments.)

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Figures

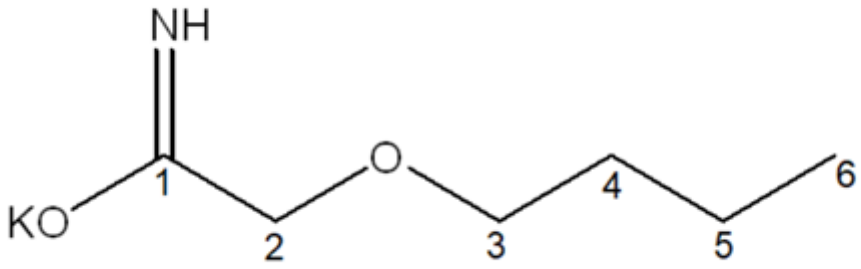


Figure 1

Structure of compound **(1)** (Potassium butoxyacetimidate)

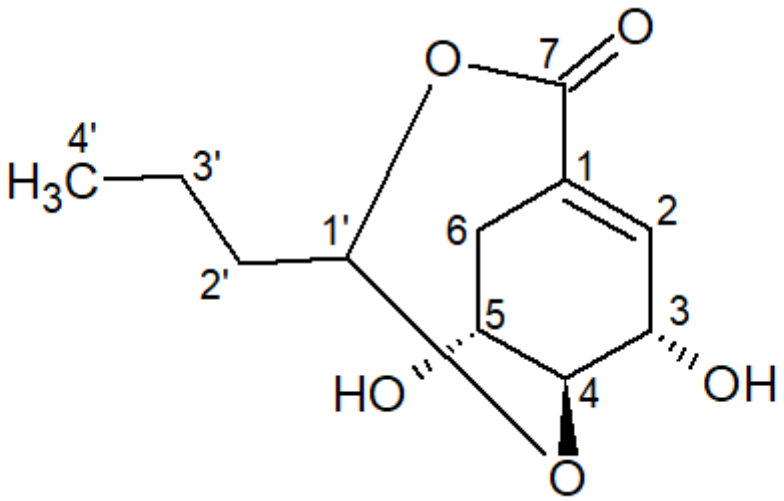


Figure 2

Structure of compound **(2)** Butyl 3(S),4(S),5(R)-4-oxy-3-epishikimic acid carboxylate acetal

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