A Khellin-like 7,7'-Glycerol-bridged Bischromone with Anti-anaphylactic Activity

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Received June 21, 1993

The synthesis of the title compound **3** is described. Its performance in Passive Cutaneous Anaphylaxis (PCA) testing was evaluated.

Ein Khellin-analoges 7,7'-glycerolverbrücktes Bischromon mit anti-anaphylaktischer Wirksamkeit

Die Synthese der Titelverbindung **3** wird beschrieben. Ihr Verhalten im PCA-Test wurde geprüft.

The structure of the well-known hay fever and asthma prophylactic, disodium cromoglycate (DNCG) **1**[1] is derived from khellin (**2**), a compound found in the Apiacea *Ammi visnaga* (L.) Lam. indigenous to the Mediterranean region. The 7,7'-glycerol-bridged bischromone **3** is even more closely related to khellin **2**, since it contains both methoxy groups as well as the oxygen atom of the furan ring of the naturally occuring compound (Scheme 1).

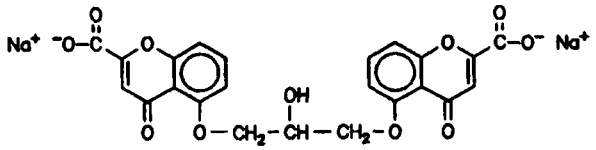
The purpose of this work is to analyze the synthesis of compound **3** and evaluate its performance in Passive Cutaneous Anaphylaxis (PCA)[2] testing.

Chemistry

The first desired intermediate product **9** is both the central compound for synthesis of the plant substances gossypetin and quercetargetin[3] as well as for the first full syntheses of khellin[4-7]. *Wessely* and *Moser*[8] used compound **9** to produce a trimethoxyflavone which - it was hoped - would help to explain the structure of the plant substance skutellarein[8].

The pathway to compound **9** begins with pyrogallol **4**, which was O-benzylated with benzyl bromide according to *Dann* and *Illing*[7] in high yield affording the pyrogalloltribenzyl ether **5** (Scheme 2).

Following *Clarke* and *Robertson*[4], quinone **7** was produced by oxidizing compound **5** with dilute nitric acid in glacial acetic acid. This yields the nitro compound **6**, which was filtered off; then again nitric acid was added to the filtrate. The product of this reaction is the quinone **7**. The density of nitric acid, *i.e.* 1.185 or 1.19, respectively, made no difference. An absolutely indispensable step turned out to be the purification of quinone **7** with tetrachloromethane, as described by *Geissmann* and *Halsall*[6]. Even when the substance has merely been recrystallized from butanone and has been judged satisfactory in spectral and other analyses,



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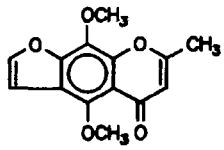

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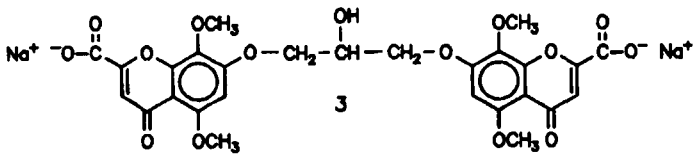

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Scheme 1

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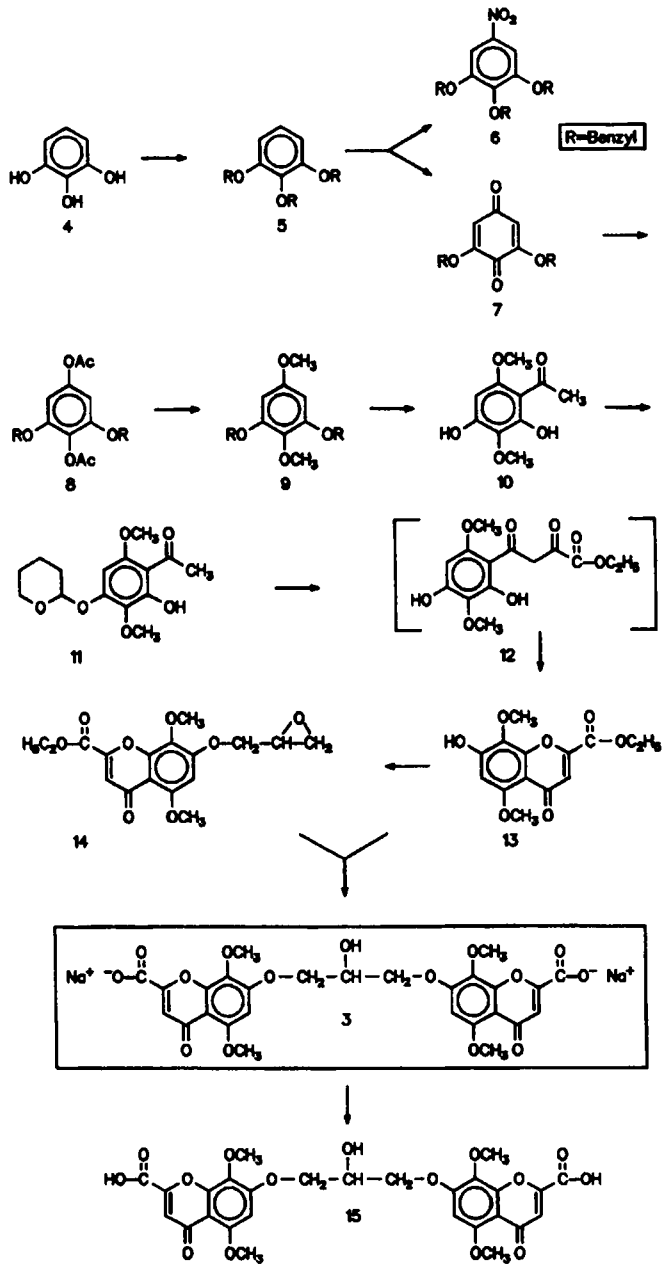

Scheme 2

even traces of the nitro compound **6** still can impede the subsequent reactions. However, a yield of approximately 30% highly purified quinone **7** can be obtained when this step is performed.

Quinone **7** was reductively acetylated in acetic anhydride in the presence of zinc powder, according to *Geissman* and *Halsall*[6]. The reaction suspension was filtered into ice water to yield a precipitate of the bisacetyl compound **8** of reagent purity.

The next step in the synthesis of compound **9**, the exchange of acetyl groups for methyl groups[6] proved to be a very problematic preparatory step. This made it necessary to convert compound **8** to the dimethoxy product **9** under thermally forced conditions by heating **8** with dimethyl sulfate in methanol for 5-8 min under reflux. Only after performing this step methanolic KOH is added at a rate of at least 180 drops/min while stirring briskly and heating. When finished, the heating mantle must be removed immediately. Compound **9** should precipitate at room temp. The yield of analytically pure crystals is 96%.

Compound **9** can be used to produce phenylethanone **10** according to *Wessely* and *Moser*, who recommend debenzylation and subsequent *Houben-Hoesch* acylation[8]. However, we assumed that, under the proper reaction conditions, it should be possible to obtain compound **10** from **9** in a single step. Therefore, $ZnCl_2$ was added to a mixture of

acetonitrile and ether and saturated with HCl gas. Compound **9** was added at 0°C. The mixture was stirred permanently at room temp. for 24 h. The supernatand was decanted and the residue was hydrolyzed with water. We were so able to increase the yield of precipitated phenylethanone **10** to approximately 90% by adding more of the catalyst.

In order to make the acetyl function of the phenylethanone **10** suitable for *Claisen* condensation with diethyl oxalate, the 4-hydroxyl function of compound **10** had first to be protected with dihydropyran by heating **10**, dihydropyran and 4-toluene sulfonic acid.

The protected compound **11** can be converted with diethyl oxalate/NaH: The not isolated oxocarbonic acid ester **12** has a strong tendency to cyclize: this ring closure associated with the formation of chromone **13** already occured when the intermediate **12** was extracted with ether from an aqueous phase (pH 1); the protective group had simultaneously been eliminated.

Chromone **13** is alkylated by epichlorohydrine in the presence of triton B, leading exclusively to the epoxide **14** (77%), which was converted to the desired product, 7,7'-glycerol-bridged bischromone **3**, by addition of chromone **13** in the presence of an ethanolic NaOH. The mixture was heated under reflux with subsequent stirring at room temp. The crude product was isolated and recrystallized from ethanol/water. The elemental analysis confirmed that compound **3** had crystallized with 4 mol of water.

Compound **3** released the dicarbonic acid **15** in 50% acetic acid as a yellow precipitate.

Biological testing of the compound 3

PCA testing was performed by Panlabs, Inc. (Table 1). Their method differs only slightly from that of *Goose* and *Blair*[2]. According to Panlabs, Inc., inhibition of > 50 is significant in PCA tests.

Table 1

COMPOUND	TEST	DOSE	RESPONSE %
3	PCA	10 mg/kg	(73)
"	"	3 mg/kg	(73) MED*
"	"	1 mg/kg	(33)
DNCG	"	5 mg/kg	(75) MED*
3	ANTIHIST.	100 mcg/ml	0
"	ANTISERO.	100 "	0

* MED = Minimal effective dose

When administered at a dose of 10, 3 and 1 mg/kg *i.v.*, compound **3** consequently inhibited the PCA response by 73, 73, and 33%, respectively. In other words, doses of 10 and 3 mg were significantly active (data in brackets), with a minimal effective dose (MED) of 3 mg/kg *i.v.*

The standard reference agent DNCG **1**, administered at 5 mg/kg *i.v.*, was used as an active control. Therefore, PCA experiments are regarded as valid only when DNCG **1** is active (inhibition is generally about 75%). The stimulating (antigenic) agent for the PCA reaction was ovoalbumin.

It would appear from these experiments that compound **3** and DNCG **1** are similarly potent and active. At lower dose levels, however, compound **3** proved superior to DNCG **1**. It should be noted that these results are "semi-quantitative", *i.e.*, groups of only three rats were used for each dose level.

Fig. 1 shows the superimposition of an acceptable (energetical) conformity of the bischromonyl compound **3** (light-gray colored) with DNCG **1** (black colored) as well as with the rigidly structured Nedocromil (Tilade^{R}, deep-gray colored).

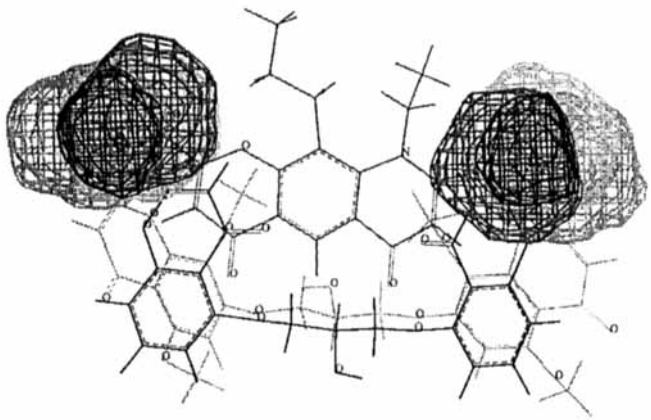

Figure 1

The superimposed volumes of the carboxylate functions also corresponded well. This confirms the efficiency of compound **3**.

We wish to thank Prof. Dr. *H.-D. Höltje* and Mrs. *K. Stahl* of our institute for valuable help with molecular modelling experiments.

Experimental Part

General Methods: Melting points: Linström apparatus, uncorrected.- IR-spectra: Perkin-Elmer 297 spectrometer. ^{1}H-NMR spectra (250 MHz): Bruker WM 250 spectrometer.- Mass spectra: Finnigan MAT Bremen CH 7A and a CH 5D spectrometer.- Elemental analyses: Institute of Pharmacy Analytical Service Laboratory.

Disodium 7,7'-[(2-hydroxy-1,3-propandiyl)bis(oxy)]bis(5,8-dimethoxy-4-oxo-4H-1-benzopyran-2-carboxylate (3)

Compounds **13** (100 mg, 0.34 mmol) and **14** (100 mg, 0.29 mmol) were dissoolved in 3 ml of a solution, prepared from 60 mg NaOH, 30 ml ethanol and 22.5 ml H_2O. The mixture was heated under reflux for 2 h and then stirred for 24 h at room temp., whereupon a pale yellow precipitate formed. The solid was separated by filtration and recrystallized from ethanol/H_2O: Pale yellow crystals, mp. 264-265°C (dec.), yield 60%.- $C_{27}H_{22}O_{15}Na_2 \cdot 4H_2O$ (704.5) Calcd. C 46.0 H 4.29 Na 6.53 Found C 45.8 H 4.55 Na 6.34.- IR (KBr): $\tilde{\nu}$ = 3428 (OH); 2940 (CH_2); 1632 cm^{-1} (C=O).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 3.81 (s; 6H, OCH_3), 3.82 (s; 6H, OCH_3), 4.22-4.38 (m; 5H, OCH_2-$CHOH$-CH_2), 5.65 (s; 1H, OH, exchangeable), 6.35 (s; 2H, 3-H, 3'-H), 6.68 (s; 2H, 6-H, 6'-H).- MS (-FAB; DMSO/glycerol): m/z = 609 (3.6; [M-23]$^-$).

1,2,3-Tribenzyloxybenzene (**5**)

Benzylbromide (513.0 g, 3 mol) was added dropwise with stirring to a solution of pyrogallol **4** (126.0 g, 1 mol) in 800 ml dry acetone. K_2CO_3 (600 g) was added and the reaction mixture was further stirred for 30 min, then it was heated under reflux for 8 h. A solution of NaOH (10 g) in 200 ml 50% methanol was added to decompose the excess of benzylbromide. After heating under reflux for 30 min inorganic salts were filtered off and the filtrate was diluted with 1 l methanol. After standing overnight at 5°C a solid product was collected and dried. An additional solid was obtained by dissolving the inorganic residue in water. The crude product was recrystallized from methanol: Colourless crystals, mp. 71°C, Lit.[7]: 71-72°C, yield 93%, Lit.[7] 91%.- $C_{27}H_{24}O_3$ (396.5) Calcd. C 81.8 H 6.10 Found C 81.8 H 6.10.- IR (KBr): 3054, 3026 (=CH); 2850 cm^{-1} (CH_2).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 4.94 (s; 2H, CH_2), 5.10 (s; 4H, CH_2), 6.64-7.55 (m; 18 H aromat.).- MS (70 eV): m/z = 396 (3.6; $M^{+\cdot}$).

2,6-Dibenzyloxy-1,4-benzoquinone (**6**)

Nitric acid (80 ml, d = 1.185) was added to a solution of **5** (160.0 g, 400 mmol) in 1.6 l glacial acetic acid. After stirring for 4 h at room temp. the yellow side product **7** was filtered off. After addition of further nitric acid (80 ml) to the filtrate the reaction mixture was allowed to stand overnight at room temp. The resulting precipitate was dried and recrystallized from butanone. To remove traces of compound **7**, the product was purified by additional heating in CCl_4: Yellow crystals, mp. 202°C, Lit.[3]: 201-202°C, yield 30%.- $C_{20}H_{16}O_4$ (320.3) Calcd. C 74.9 H 5.03 Found C 74.8 H 4.96.- IR (KBr): $\tilde{\nu}$ = 3059-3025 (=CH); 1696, 1641 (C=O); 1624 cm^{-1} (C=C).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 5.02 (s; 4H, CH_2), 5.86 (s; 2H, 3-H, 5-H), 7.36 (m; 10 H aromat.).- MS (70 eV): m/z = 320 (14; $M^{+\cdot}$).

2,6-Dibenzyloxy-1,4-hydroquinone-diacetate (**8**)

A mixture of **6** (16.0 g, 50 mmol), sodium acetate (8.0 g) and 120 ml acetic anhydride was heated under reflux for 30 min. During this time zinc powder (13.6 g) was added portionwise. The reaction mixture was further refluxed for 30 min and then filtrated. Additional product was recovered by elution of the residue with glacial acetic acid. The combined filtrates were poured into ice water. The resulting precipitates were collected, washed with water and dried. The product was analytically pure: Colourless crystals, mp. 148°C, Lit.[6]: 147-148°C, yield 99%, Lit.[6] "quantitative".- $C_{24}H_{22}O_6$ (406.4) Calcd. C 70.9 H 5.46 Found C 70.5 H 5.51.- IR (KBr): 2919 (CH_2); 1762 cm^{-1} (C=O).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.24 (s; 6H, $COCH_3$), 5.04 (s; 4H, CH_2), 6.45 (s; 2H, 3-H, 5-H), 7.35 (m; 10 H aromat.).- MS (80 eV): m/z = 406 (9; $M^{+\cdot}$).

2,6-Dibenzyloxy-1,4-dimethoxybenzene (**9**)

Compound **8** (7.4 g, 18.2 mmol) was dissolved in 100 ml methanol and 20 ml dimethyl sulfate. The mixture was heated under reflux for 5 min, then a solution of KOH (22.4 g) in 80 ml methanol/H_2O (1:1) was added dropwise (180 dr/min) to the hot solution. After cooling to room temp., the precipitated solid was washed with water and dried. The product so obtained was analytically pure: Colourless crystals, mp. 78.5°C, Lit.[6]: 80°C, yield 96%, Lit.[6]: 79%.- $C_{22}H_{22}O_4$ (350.4) Calcd. C 75.4 H 6.33 Found C 75.8 H 6.31.- IR (KBr): $\tilde{\nu}$ = 2917 cm^{-1} (CH_2).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.66 (s; 3H, CH_3), 3.81 (s; 3 H, CH_3), 5.10 (s; 4H, CH_2), 6.19 (s; 2H, 3-H, 5-H), 7.29-7.54 (m; 10 H aromat.).- MS (80 eV): m/z = 350 (25; $M^{+\cdot}$).

1-(2,4-Dihydroxy-3,6-dimethoxy)phenylethanone (**10**)

$ZnCl_2$ (2.0 g) was added to a solution of 25 ml acetonitrile and 35 ml dry ether. The mixture was saturated with HCl gas. After cooling to 0°C compound **9** (2.7 g, 7.7 mmol) was added and the mixture was stirred at room temp. for 24 h. The supernatand was decanted and the residue was triturated with 50 ml water and heated for 3 h on the water bath. After cooling to 5°C the precipitate was collected and four times recrystallized from petroleum ether (bp. 100-140°C): Colourless crystals, mp. 128°C, Lit.[8]: 129°C, yield 92%.- $C_{19}H_{12}O_5$ (212.2) Calcd. C 56.6 H 5.76 Found C 57.0 H 5.69.- IR (KBr): $\tilde{\nu}$ = 3386 (OH); 2945 (CH_3); 1629 cm^{-1} (C=O).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 2.53 (s; 3H, $COCH_3$), 3.65 (s; 3H, OCH_3), 3.80 (s; 3H, OCH_3), 6.04 (s; 1H aromat.), 10.22 (s; 1H, OH, exchangeable), 13.90 (s; 1H, OH, exchangeable).- MS (80 eV): m/z = 212 (98; $M^{+\cdot}$).

1-[2-Hydroxy-3,6-dimethoxy-4-(tetrahydropyran-2-yloxy)]phenylethanone (**11**)

A mixture of **10** (1.0 g, 4.7 mmol), dihydropyrane (10 ml) and a trace of 4-toluene sulfonic acid was heated at 60°C for 5 min, then stirred for 24 h at room temp. The mixture was poured into water and extracted with ether (3 x 30 ml) at pH 11. The org. extract was dried over Na_2SO_4. Evaporation of the solvent gave a syrupy product that was dried on a clay disk: Yellow crystals, mp. 115°C, yield 68%.- $C_{15}H_{22}O_6$ (296.3) Calcd. C 60.8 H 6.80 Found C 60.9 H 6.92.- IR (KBr): $\tilde{\nu}$ = 3427 (OH); 1622 cm^{-1} (C=O).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.63-2.09 (m; 6H, CH_2), 2.62 (s; 3H, $COCH_3$), 3.66 (m; 1H, OCH_2), 3.84 (s; 3H, OCH_3), 3.86 (s; 3 H, OCH_3), 3.89 (m; 1H, OCH_2), 5.60 (br; 1H, OCHO), 6.26 (s; 1H, 5-H), 13.83 (s; 1H, OH, exchangeable).- MS (70 eV): m/z = 296 (2.5; $M^{+\cdot}$).

Ethyl 7-hydroxy-5,8-dimethoxy-4-oxo-4H-1-benzopyran-2-carboxylate (**13**)

A solution of **11** (0.75 g, 2.5 mmol) in dry diethyl oxalate (20 ml) was added dropwise to a suspension of NaH (1.0 g) in diethyl oxalate (30 ml) at room temp. After stirring for 10 h the mixture was diluted with H_2O, acidified with HCl (pH 1) and extracted with ether (3 x 50 ml). The org. layers were separated and dried over Na_2SO_4. Evaporation gave a solid residue that was recrystallized from ethanol: Yellow crystals, mp. 220°C, yield 68%.- $C_{14}H_{14}O_7$ (294.3) Calcd. C 57.1 H 4.80 Found C 56.9 H 4.81.- IR (KBr): $\tilde{\nu}$ = 3085 (OH); 1737, 1642 cm^{-1} (C=O).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 1.35 (t; J = 7 Hz, 3H, CH_3), 3.78 (s; 3H, OCH_3), 3.82 (s; 3H, OCH_3), 4.38 (q; J = 7 Hz, 2H, CH_2), 6.52 (s; 1H, 3-H), 6.69 (s; 1H, 6-H), 10.98 (s; 1H, OH, exchangeable).- MS (80 eV): m/z = 294 (66; $M^{+\cdot}$).

Ethyl 7-(2,3 epoxypropoxy)-5,8-dimethoxy-4-oxo-4H-1-benzopyran-2-carboxylate (**14**)

Compound **13** (100 mg, 0.34 mmol) was dissolved in 10 ml epichlorohydrine and 2 drops of triton B. The mixture was heated for 24 h at 70°C. Evaporation gave a product of high purity. Recrystallization from ethanol gave colourless crystals, mp. 144°C, yield 75%.- $C_{17}H_{18}O_8$ (350.3) Calcd. C 58.3 H 5.18 Found C 58.2 H 5.18.- IR (KBr): $\tilde{\nu}$ = 2938 (CH_2); 1728, 1664 cm^{-1} (C=O).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 1.34 (t; J = 7 Hz, 3H, CH_3), 2.77 (dd; 1H, J_1 = 2.3 Hz, J_2 = 5 Hz, oxiran-CH_2), 2.90 (t; J = 5 Hz, 1H, oxiran-CH_2), 3.42 (m; 1H, oxiran-CH), 3.83 (s; 3H, OCH_3), 3.86 (s; 3H, OCH_3), 4.09 (dd; J_1 = 12 Hz, J_2 = 7 Hz, 1H, CH_2O), 4.37 (q; J = 7 Hz, 2H, CH_2), 4.62 (dd; J_1 = 12 Hz, J_2 = 2.3 Hz, 1H, OCH_2), 6.67 (s; 1H, 3-H), 6.74 (s; 1H, 6-H).- MS (70 eV): m/z = 350 (100; $M^{+\cdot}$).

7,7'-[(2-Hydroxy-1,3-propandiyl)bis(oxy)]bis(5,8-dimethoxy-4-oxo-4H-1-benzopyran-2-carboxylic acid) (**15**)

A solution of compound **3** (100 mg, 0.14 mmol) in 8 ml 50% acetic acid was refluxed for 30 min. The solution was then stirred for 24 h at room temp. The solid product was washed with 20 ml of 70% ice cold acetic acid. The yellow crystals were analytically pure, mp. 235°C, yield 67%.- $C_{27}H_{24}O_{15} \cdot 2H_2O$ (624.5) Calcd. C 51.9 H 4.48 Found C 51.7 H 4.00.- IR

(KBr): $\tilde{\nu}$ = 3433 (OH); 2936 (CH_2); 1729, 1649 cm^{-1} (C=O).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 3.80 (s; 6H, OCH_3), 3.86 (s; 6H, OCH_3), 4.22-4.39 (m; 5H, OCH_2-$CHOH$-CH_2O), 5.67 (s; 1H, OH, exchangeable), 6.59 (s; 2H, 3-H, 3'-H), 6.75 (s; 2H, 6-H, 6'-H).- MS (70 eV): m/z = 588 (1.5; $M^{+\cdot}$).

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