

SHORT COMMUNICATIONS

Isolation and Synthesis of 2-Phenyl Benzopyranones and Comparative Evaluation of Their Smooth Muscle Relaxant Activity

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Accepted 6 June 2000

Revised 6 May 2000

Received 5 November 1999

A few 2-phenyl benzopyranones (substituted flavanones) were isolated from the available sources or synthesized by simple base catalyzed condensation of appropriate aryl aldehydes and substituted 2'-hydroxy acetophenones. The effect of these substituted flavanones on contraction evoked by oxytocin, on rat uterus was studied. Of the isolated/synthesized compounds, naringenin showed maximum inhibition of oxytocin-induced contraction of rat uterus.

Naturally occurring flavanones with different substitution patterns have been shown to possess many biological activities including smooth muscle relaxant effect¹. Sakuranetin obtained from *Dodonea viscosa* (Sapindaceae) has been found to exhibit smooth muscle relaxant activity¹. The antispasmodic actions of naringenin and eriodictyol isolated from *Satureja obovata* have been reported². The above reports indicate that the flavanones possess antispasmodic activity. The present study was conducted to evaluate the effect of substituted flavanones (hesperidin, naringenin, pinocembrin, liquiritigenin) on the contractions evoked by oxytocin in rat uterus.

Chemical structure of isolated/ synthesized compounds are given in Table 1. Defatted and dried orange peel powder³ (450 g) was refluxed with 95% ethanol (4 l x 3, 3 h). The combined ethanol extract was concentrated *in vacuo* and then evaporated on a water bath to get a semisolid mass. The crude mass, after washing with acetone (25 ml) 4 to 5 times and once with chloroform (25 ml), was crystallised from methanol as colourless needles. (Hesperidin) (yield: 0.18%); m.p. 254 - 256°

U.V λ max in nm for the compound isolated from orange peels: MeOH-284, 328, (+NaOMe)-242, 286, 355, (+AlCl₃)-307, 385 (+AlCl₃/HCl)-306, 382, (+NaOAc)-284, 328, and (+NaOAc/H₃BO₃) 284, 328, while, for hesperidin⁴ these shifts were: MeOH-283, 326, (+NaOMe)- 242, 286, 356, (+AlCl₃)- 308, 383, (+AlCl₃/HCl)-306, 379, (+NaOAc)-284, 328 and (+NaOMe)-284, 326.

IR(KBr) cm⁻¹: 3490 (bonded OH str.); 1640 (C = O str.); 1630 (aromatic C = C str.); 740, 770, 810, 1270, 1450, 2980.

Defatted powdered *Citrus decumana* peels³ (400 g) were extracted with 95% ethanol (4l x 2; 3h) in a 5 l round bottomed flask under reflux. The combined extract was concentrated *in vacuo*. The syrupy mass was then extracted with solvent ether (50 ml x 3), followed by ethyl acetate. The combined ethyl acetate extract was concentrated, freed from the solvent by evaporation and then taken up in methanol (2 ml). Naringin crystallised from methanol on keeping. The crude naringin was hydrolysed with acid to give its aglycone naringenin. (yield: 0.1%); m.p. 225 - 227°

U.V λ max in nm for the compound isolated from *Citrus decumana*: (MeOH)- 289, 326, (+NaOMe)- 245, 323, (+AlCl₃)- 312, 375, (+AlCl₃/HCl)- 311, 371,

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(+NaOAc)- 284sh, 323 (+NaOAc/H₃BO₃)-290, 332sh. These shifts for naringenin⁴ were: (MeOH)- 289, 326sh (+NaOMe)- 245, 323, (+AlCl₃)- 312, 375, (+AlCl₃/HCl)- 311, 371, (+NaOAc)- 284sh, 323, (+NaOAc/H₃BO₃)-290, 332sh.

IR (KBr) cm⁻¹: 3649.1 (bonded O-H str.); 1643 (C = O str.); 1602.7 (aromatic C = C str.); 731, 800, 831, 1249, 1461, 2918.

Powdered Shade dried leaves of *Elytranthe parasitica*³ (2 kg) were extracted under reflux with (95%) ethanol (3 l x 3; 4h). The combined extract was concentrated under reduced pressure and then evaporated to a semisolid consistency. The dried residue was suspended in distilled water (1l) and was extracted with 3 portions of 500 ml each of ethyl acetate. Extract was dried over anhydrous sodium sulphate bed and evaporated to dryness. The residue was dissolved in ethyl acetate (50 ml) containing a few drops of methanol and was loaded on a column of silica-gel (400 g). It was eluted with 1%, 2% and 5% methanol in ethyl acetate. Each fraction was subjected to TLC (silica-gel) [Solvent system- ethyl acetate: Benzene (1:1)]. Identical fractions were pooled together and concentrated to yielded a colourless compound which was recrystallised from acetone (Pinocembrin) (yield: 0.05%); m.p. 113 - 114^o

U.V λ max in nm for the compound isolated from *Elytranthe parasitica* are:

MeOH-284, 328sh, (+ NaOMe)- 243, 326, 351, (+AlCl₃)- 309, 381, (+AlCl₃/HCl)- 309, 381, (+NaOAc)- 284, 329, NaOAc/H₃BO₃ 283,329. These shifts for pinocembrin⁴ were: MeOH- 289, 325sh, (+ NaOMe)-245, 324, (+AlCl₃)- 311, 375, (+AlCl₃/HCl)-309, 373, (+NaOAc)- 253sh, 283, 323, (+NaOAc/H₃BO₃)-291, 326sh¹.

IR (KBr) cm⁻¹: 3384 (bonded O-H str.); 1646 (C = O str.); 1576 (aromatic C = C str.); 770, 819, 1290, 1444.8, 2918.

A solution of 2,4-dihydroxyacetophenone (Aldrich Chemicals Inc., U.S.A.) (0.01 mol) in ethanol (4 ml) was added to 4 ml of 50 % sodium hydroxide solution in portions with stirring to get a homogeneous paste. To this paste was added dropwise a solution of p-hydroxybenzaldehyde (S.D. Fine Chemicals., Mumbai) (0.015 mol) in ethanol (3 ml) with constant stirring till a clear solution was obtained. The reaction mixture after keeping overnight was decomposed with 80 ml of dilute hydrochloric acid containing 8 ml of concentrated hydro-

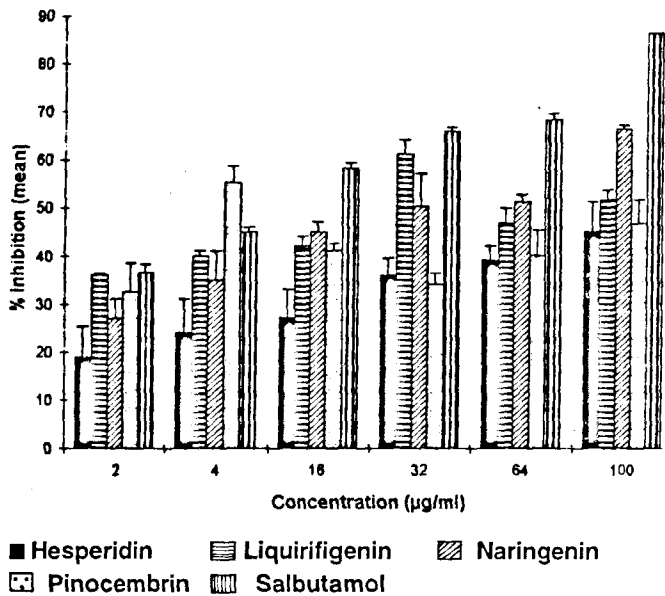


Fig 1: Inhibition of oxytocin-induced contraction of rat uterus

Mean percent inhibition of rat uterus contraction induced by oxytocin by hesperidin, naringenin, pinocembrin and liquiritigenin in comparison to the inhibition produced by standard salbutamol.

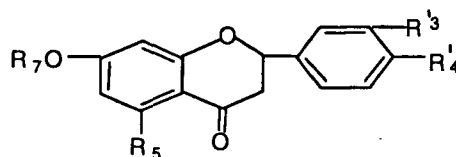
chloric acid. After usual work-up, the desired chalcone was obtained⁵. The chalcone (Isoliquiritigenin) obtained was purified by column chromatography using silica gel of chromatographic grade. Petroleum ether (60–80^o):ethyl acetate (2:1) mixture was used for elution.

To a solution of isoliquiritigenin (0.01 mol) in methylene dichloride (30 ml), silica gel (chromatographic grade 6 g) and 3 drops of concentrated sulphuric acid were added. The mixture was stirred on a magnetic stirrer for 6 h and silica gel was filtered off. The solvent was distilled off. The corresponding flavanone, liquiritigenin was separated by fractional crystallization from ethanol⁶. (yield: 38%); m.p. 200-202^o.

U.V λ max in nm for the synthesised compound are: MeOH-272, 312, (+NaOMe)- 242, 328, (+AlCl₃)- 284, 355, (+AlCl₃/HCl)- 384, 355, (+NaOAc)- 280, 300, 329sh (+NaOAc/H₃BO₃)-276, 315. These shifts for liquiritigenin⁴ were: MeOH-276, 312, (+NaOMe)- 250, 327sh, (+AlCl₃)- 276, 311, (+AlCl₃/HCl)- 276, 311, (+NaOAc)-255, 282, 327sh, (+NaOAc/H₃BO₃)-335.

IR (KBr) cm⁻¹ : 3380 (bonded O-H str.); 1640 (C = O str.); 1570 (aromatic C = C str.); 770, 830, 1290, 1460, 2940.

TABLE 1: CHEMICAL STRUCTURES OF ISOLATED/SYNTHESIZED COMPOUNDS



Compound	R ₇	R ₅	R ₃ '	R ₄ '
Hesperidin	Rhamnoglucosyl -	OH	OH	OCH ₃
Naringenin	H	OH	OH	OH
Liquiritigenin	H	H	H	OH
Pinocembrin	Glucosyl -	OH	OH	H

Six young female Wistar rats with body weights between 120-150 g were used for the experiment. Each animal was injected with estradiol benzoate (800 µg/kg body weight in ethanol) i.m., 48 h prior to the experiment. The animal was killed by stunning and the abdomen was opened quickly. The bicornuate uterus was carefully removed and was placed in a shallow dish containing De Jalon's solution and dissected free of fat and ovaries. Care was taken not to stretch the preparation. One horn was then mounted in an isolated organ bath in De Jalon's solution at 31° aerated with air. A period of 15 min was allowed for stabilization with renewal of perfusing fluid every 10 min. Recording of the response was done using frontal writing lever balanced for a tension of about 1 g. The data obtained from all the six isolated uterine experiments were expressed as mean ± S.D, analysed using student's 't'-test. Level of significance was fixed at p < 0.05.

Naringenin showed maximum inhibition (66%) of oxytocin-induced contraction of rat uteri in comparison to other test compounds (hesperidin, pinocembrin, liquiritigenin). Thus it may be worthwhile to synthesize compounds related to naringenin and to study the SAR by employing other screening models for smooth muscle relaxant activity.

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