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Full Length Research Paper

Bioactive chalcone from *Indigofera pulchra*

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Extensive phytochemical investigations using silica gel column chromatography, gel filtration and preparative thin layer chromatography (TLC) led to the isolation of 2' 4'- dihydroxy-4-prenyloxy chalcone from the methanol extract of *Indigofera pulchra*. The structure of this compound was elucidated with the help of NMR and mass spectroscopy. The analgesic effect of the compound was studied using acetic acid induced writhing and hot plate tests in mice. The compound isolated showed significant analgesic activity in acetic acid induced writhing test but no activity in the hot plate test, suggesting that it posses peripheral analgesic activity. To the best of our knowledge, this is the first report of the isolation of this compound.

Key words: Prenyloxychalcone, analgesic activity, *Indigofera pulchra*, 1 and 2D NMR.

INTRODUCTION

Indigofera pulchra (Willd): Papilionaceae is an annual non climbing herbs or shrub that can grow up to 1 m tall. It is widely distributed throughout tropical and subtropical regions of Nigeria, Niger, Togo, Benin, Ghana and Chad (Hepper, 1976). In ethnomedicine, the leaves are used to treat infected wound (Herper, 1976; Burkill, 1995), while the decoction of the aerial part is used as prophylactic against snake-bite (Sule et al., 2003), analgesic and anti-inflammatory (Abubakar et al., 2007) and to treat gastrointestinal pain. The decoction is also used to counteract various poisons (Burkill, 1995) and is effective against malaria and dysentery (Adamu et al., 2005; Asase et al., 2005). As part of our research project focusing on Nigerian medicinal plants, here we report the isolation of a new prenyloxychalchone from *I. pulchra* and its analgesic activity.

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MATERIALS AND METHODS

Collection, Identification and Preparation of plant materials

The whole plant (*I. pulchra*) growing wild was collected from Samaru-Zaria, Kaduna State, Nigeria in the month of September, 2007 and was authenticated at the Herbarium, Biological Sciences Department, Ahmadu Bello University Zaria, Nigeria, where a voucher specimen (No. 410) was deposited for future reference. The aerial part was cut, air-dried to constant weight and size reduced using pestle and mortar and subsequently referred to as powdered plant material.

Extraction

The powdered plant material (1000 g) was extracted with methanol (2 L) using Soxhlets apparatus, the solvent was removed *in-vacuo* to yield a residue (178 g) referred to as *I. pulchra* methanol extract. The methanol extract (60 g) was treated successively with hexane,

Table 1. Eluates obtained from column chromate-graphy of ethylacetate fraction.

Fraction	Number of spots
1	3
2	6
3	5
4	3
5	4
6	5
7	6

ethylacetate and acetone to afford hexane (7.3 g), ethylacetate (5.2 g) and acetone (10.2 g) fractions respectively.

Column chromatography of ethylacetate fraction

Ethylacetate fraction (4 g) was chromatographed over silica gel packed column of dimension 75 by 3.5 cm, the column was eluted continuously using dichloromethane: ethylacetate (30:1) as solvent system. Eighty fractions, 30 ml each were collected. The fractions were pooled together based on their thin layer chromatography (TLC) profile to give five major fractions and the column was finally washed with methanol to give the sixth fraction. Fraction 3 which showed one major spot and two minor spots was further subjected to preparative thin layer chromatography using chloroform: ethyl acetate (19:1) as the solvent system. The major spot was scraped; the adsorbent was thoroughly washed with ethyl acetate. The solution was concentrated to afford yellow amorphous compound coded D5 (20 mg). D5 was subjected to chemical and spectroscopic analysis to elucidate its chemical structure.

Spectral analysis

Proton and carbon-13 NMR spectra

NMR spectra (both 1D and 2D) were obtained on a Bruker AVANCE (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer, using the residual solvent peaks as internal standard. Chemical shift values (δ) were reported in parts per million (ppm) relative to appropriate internal solvent standard and coupling constants (*J* values) are given in Hertz. HMBC spectra were optimized for a long range $J_{H\text{-}C}$ of 7Hz (d_6 = 0.07s). The NMR solvents used for these measurements are deutrated chloroform and deutrated benzene. Mass spectra were recorded on a JEOL JMS-AX505HA double-focusing instrument at 70 eV

Acetic acid – induced writhing test in mice

The method previously described by Koster et al. (1959) was used. Twenty five Swiss Albino mice of either sex were divided into five groups each containing five mice. Group I was injected with 10 ml/kg normal saline (negative control). Groups II, III and IV were injected intraperitonaeally with 2.5, 5 and 10 mg/kg of the compound respectively. Group V was injected with ketoprofen 10 mg/kg (positive control). 30 min later, each mouse was treated with 10 ml/kg of aqueous solution of acetic acid (0.6%) intra-peritonaeally. The number of abdominal writhings for each mouse was counted for a period of ten minutes using tally counter after five minutes latency. The percentage inhibition of abdominal writhings was calculated using the following formula.

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of writhes (Control)} - \text{Mean No. of writhes (test)}}{\text{Mean No. of writhes (Control)}} \times 100$$

Hot plate test method

The method of Turner (1965) as modified by Lanhers et al. (1992) was adopted. Mice were placed on a thermostated hot plate maintained at temperature of 50 ± 1°C. The time taken for the mice to leak their paw or jump off the hot plate was considered as pain reaction time and was recorded for each mouse. Mice that showed initial pain reaction response within 30 s were selected and used for the study. The mice were then divided into 5 groups of 5 mice per group. Group I served as negative control and received 10 ml/kg of 0.9% normal saline (vehicle) while Groups II, III and IV received the compound (*i.p.*) at dose 2.5, 5 and 10 mg/kg, respectively and the fifth group received morphine (5 mg/kg). Thirty minutes later, each mouse was placed on a hot plate and the pain reaction time recorded.

Statistical analysis

The result of the acetic acid induced writhing and hot plate tests were expressed as Mean ± SEM and were analysed using one way ANOVA followed by Dunnett post hoc test for multiple comparisons. Differences were regarded as significant when $P<0.05$.

RESULTS

Isolation of D5

Silica gel column chromatographic separation of the ethylacetate soluble portion of the methanol extract yielded seven fractions when eluted with 30:1 dichloromethane: ethylacetate (Table 1).

Preparative thin layer chromatography on fraction 4 which showed one major spot (R_f = 0.44) and two minor spots (R_f = 0.16 and 0.09) led to the isolation of a yellow amorphous compound (15 mg) coded D5.

Thin layer chromatographic analysis of D5

Thin layer chromatographic (TLC) analysis of D5 using Chloroform: ethylacetate (19:1) and (2:3) revealed single homogeneous spot with R_f values of 0.36 and 0.89, respectively. When the spots were exposed to ammonia vapor and viewed under UV light dark red fluorescence was observed.

Solubility of D5

D5 was found to be soluble in acetone, ethylacetate and methanol.

Melting point of D5

The sample was found to have a melting point range of

Table 2. NMR DATA of D5.

Position	DEPT	δC	δH, J in Hz
1	C	129	-
2, 6	CH	131.5	7.7, 8.5, 2.0 Hz
3, 5	CH	116.2	6.98, 8.5 Hz
4	C	162.7	-
1’	C	114.8	-
2’	C	166.5	-
3’	CH	103.6	6.30, 2.0
4’	C	167.5	-
5’	CH	108.9	6.43, 2.0
6’	CH	133.4	7.98
1’’	CH_2	66.1	4.60
2’’	CH	120.8	5.47
3’’	C	139.2	-
4’’	CH_3	17.5	1.77
5’’	CH_3	25.5	1.80
1’’’	C = O	193.5	-
2’’’	CH	119.3	7.66
3’’’	CH	145.2	7.81

138 to 142°C.

Chemical tests on D5

D5 produced red colouration when subjected to Shinoda test, brown precipitate when treated with ferric chloride solution and dissolve in concentrated sulphuric acid to give red colour solution.

Spectral analysis of D5

Proton magnetic resonance of D5

The ^{1}HNMR spectra of D5 exhibited signals for meta-coupled (J = 2.0 Hz) aromatic hydrogens (δ 6.30, 1H; 6.43, 1H), ortho-coupled (J = 9.0 Hz) hydrogens (δ 7.99 1H; 6.43, 1H) and an AA’BB’ aromatic splitting pattern (6.98, 2H, t, J = 9 Hz; 7.7, 2H, dd, J = 9.2, 2.0 Hz). It also revealed the presence of two hydrogens J = 15.0 Hz (δ 7.81, 1H; 7.66, 1H), this is typical of olefinic hydrogens in trans arrangement. Other resonances were at δ 4.60, 2H, J = 7.0 Hz, 5.47, 1H, 1.77, 3H, s and 1.80, 3H, s indicating the presence of prenyloxy moiety as a side chain of the molecule (Table 2).

Carbon-13 magnetic resonance of D5

The ^{13}C and distortion less enhancement by polarization transfer (DEPT) NMR spectra of D5 disclosed two carbons singlets for methyl groups, one methylene , nine aromatic methine carbons and eight quaternary aromatic carbons (Table 2).

The HMQC spectra of D5 were used to assign protons on their respective carbons. The HMBC spectra were used to establish the connectivity between different fragments of the molecule (Table 3). Based on the result of chemical tests, 1D, 2D NMR and MS D5 was assigned the following structure (Figure 1).

Effect of chalcone (D5) on acetic acid-induced abdominal constrictions in mice

The compound at doses of 2.5, 5 and 10 mg/kg significantly (P<0.05) reduced the number of acetic-acid-induced abdominal writhes by 71.40, 68.4 and 82.7%, respectively. Ketoprofen (10 mg/kg) produced 88.0% reduction in abdominal constriction (Table 4).

Effect of the chalcone on pain reaction time in hot plate test in mice

In the hot plate test, the compound at the doses tested did not significantly (P<0.05) increased the pain reaction time, while morphine the standard analgesic agent used significantly increased the pain reaction time (Table 5).

DISCUSSION

Column chromatography of the ethyl acetate fraction

Table 3. HMBC data of D5.

Position	δC	HMBC
1	129.0	2’’’, 3’’’, 3, 5
4	162.7	2, 6, 3, 5
1’	114.8	3’, 5’
2’	166.5	3’, 6’
4’	167.5	6’, 3’
5’	108.9	3’
2’’	120.8	4’’, 5’’, 1’’
3’’	139	4’’, 5’’, 1’’
4’’	17.5	5’’
5’’	25.5	4’’
1’’’	193.5	2’’’, 3’’’, 6’
2’’’	119.3	3’’’
3’’’	145.2	2, 6, 2’’’

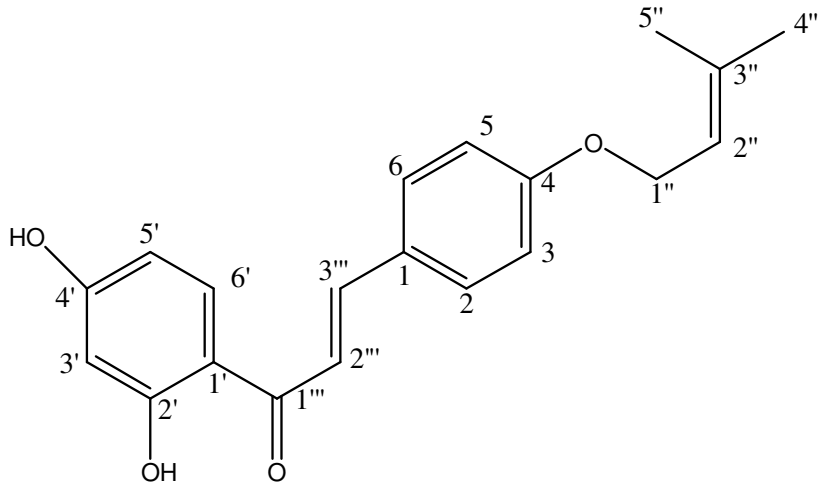

Figure 1. 2’ 4’- dihydroxy-4-prenyloxy chalcone.

Table 4. Effect of the chalcone on acetic acid-induced abdominal writhing in mice.

Treatment	Dose	Mean number of writhes ± SEM	Inhibition (%)
Normal saline	10 ml/kg	26.60 ± 3.1	-
Ketoprofen	10 mg/kg	3.2 ± 1.6[a]	88.0
D5	2.5 mg/kg	7.6 ±1.5[a]	71.4
D5	5 mg/kg	8.4 ± 2.0[a]	68.4
D5	10 mg/kg	4.6 ± 1.1[a]	82.7

[a] $P<0.05$; (compared with control); Dunnet post hoc t-test for multiple comparison.

followed by preparative thin layer chromatography led to the isolation of D5. The positive chemical reactions shown by the compound when subjected to Shinoda and ferric chloride tests gave an indication that the compound has a flavonoid nucleus with some free phenolic hydroxyl groups (Silva et al., 1998). The red coloured solution formed when the compound was dissolve in concentrated sulphuric acid implies that the compound is likely to be a

Table 5. Effect of the chalcone on pain reaction time in hot plate test in mice.

Treatment/ Dose	Pain reaction time (s) ± SEM
Normal saline (10 ml/kg)	1.33 ± 0.15
D5 (2.5 mg/kg)	1.5 ± 0.15
D5 (5 mg/kg)	1.2 ± 0.06
D5 (10 mg/kg)	1.63 ± 0.24
Morphine (5 mg/kg)	16.8 ± 0.38[a]

a = significant at P<0.05 - significantly different compared to negative control.

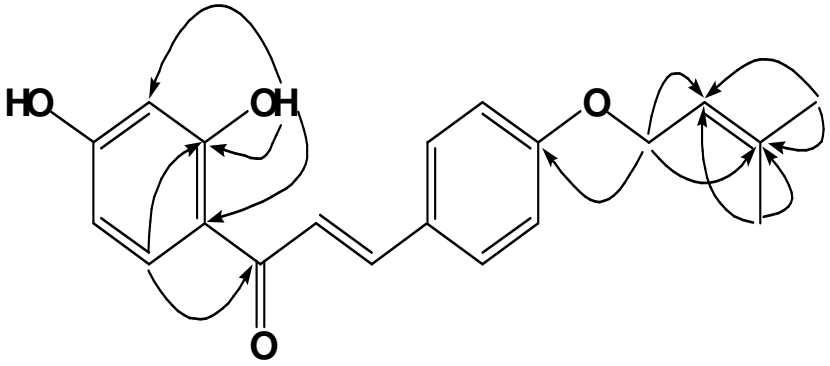

Figure 2. Selected HMBC correlations of D5.

chalcone or an aurone (Silva et al., 1998). The dark red florescence produced by the compound when exposed to ammonia vapour and viewed under UV light indicates the presence of chalcone nucleus (Harborne, 1973). The molecular formula of the compound (D5) was assigned as $C_{20}H_{20}O_4$ from an $[M+H]^+$ peak at m/z 325.1827 in the high-resolution EIMS with a base peak at m/z 256 which is due to the loss of isoprenyl side chain other peaks were observed at 137 and 120.

The ^{1}HNMR spectrum of D5 exhibited signals for meta-coupled (J = 2.0 Hz) aromatic hydrogens (δ 6.30, 1H; 6.43, 1H), ortho-coupled (J = 9.0 Hz) hydrogens (δ 7.99 1H; 6.43, 1H), due to 1, 2, 4, trisubstituted benzene and an AA'BB' aromatic splitting pattern (6.98, 2H, t, J = 9 Hz; 7.7, 2H, dd, J = 9.2, 2.0 Hz) indicating a 1, 4, disubstituted benzene. It also revealed the presence of two hydrogens J = 15.0 Hz (δ 7.81, 1H; 7.66, 1H), this is typical of olefinic hydrogens in trans arrangement. Other resonances at δ 4.60, 2H, J = 7.0 Hz due to oxymethylene, 5.47, 1H, due to methine hydrogen,1.77, 3H, s and 1.80, 3H, s representing two methyl groups are indicative of the presence of a prenyl moiety as a side chain of the molecule (Fortin et al., 2001; Lee et al., 2003).

The ^{13}C and DEPT NMR spectra of D5 revealed signals at δ 66.1, 120.8, 139, 17.5 and 25.5, these carbon signals are typical of prenyloxy side chain (Fortin et al., 2001). Also observed is a signal at 193.5 which is due to carbonyl carbon, other resonances observed were for nine aromatic methine and five quaternary aromatic carbons, these resonances suggest presence of chalcone nucleus. The assignment of carbons and the placement of the prenyloxy side chain were achieved using two dimensional NMR experiment.

In the HMBC spectrum, a common ^{3}J correlations between hydrogen (H3') at δ 6.30 and that at δ 6.43 (H5') to a quaternary carbon δ 114.8 confirmed its assignment as C1', also a common ^{3}J correlations between 3, 5 hydrogen δ 6.98 and H2''' (δ 7.66) to a quaternary carbon δ 129 confirmed its assignment as C1 and also suggest that the chain of olefinic carbons is attached to ring B at C1. A^{3}J correlation observed between hydrogen at 6'H, δ7.98 and quatenary carbonyl carbon confirmed that C = O is linked to C-1' of ring A.

In the prenyloxy side chain the methyl hydrogens showed ^{3}J correlation to the methyl carbons of their respective partners and to a methine carbon δ 120.8, they also show ^{2}J correlation to the quaternary carbon (C3'', δ139) on which they are attached. The oxymethylene hydrogens δ 4.60 of the prenyloxy side chain showed ^{3}J correlation to oxygen bearing quaternary carbon (C4, δ 162.7, this indicate that the prenyloxy side chain is joined to the molecule through C4 carbon (Figure 2).

Based on the results of chemical tests, mass spectroscopy, 1D and 2D NMR spectral analysis, D5 was

suggested to be prenylated chalcone, 2', 4'dihydroxy, 4 prenyloxy chalcone.

D5 is structurally similar to isoliquiritegenin which is a 2', 4', 4- trihydroxychalcone isolated from *Glycerrhiza ularensis* roots, it can be considered to be a derivative of isoliquiritegenin in which the hydroxyl group (OH) at position 4 is substituted with a prenyloxy side chain. Isoliquiritegenin has been reported to posses antispasmodic (Sato et al., 2007), selective histamine-2 receptor inhibition (Kim et al., 2006), vasorelaxant, anti-platelet aggregation and anticancer (Takahashi et al., 2004) activities.

The ability of the compound to significantly reduce the number of acetic acid induced writhes suggests that it possessed peripherally mediated analgesic activity. Acetic acid-induced abdominal constriction test is used for the evaluation of peripheral analgesic activity (Gene et al., 1998). The abdominal constriction response is thought to involve in part local peritoneal receptors (Bentley et al., 1983), it is therefore possible to suggest that the mechanism of action of the compound may be linked to interference with peritoneal receptors and cyclooxygenases and /or lipooxygenases (Deradt et al., 1980; Levini et al., 1984). Since the compound did not significantly increase the pain reaction time in the hot plate test, it can be suggested that it does not posses centrally mediated analgesic activity.

REFERENCES

Abubakar MS, Musa AM, Ahmed A, Husaini IM (2007). The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribes of Northern Nigeria. J. Ethnopharmacol., 111(3): 625-629.

Adamu HM, Abayeh OJ, Agbo MO, Abdullahi AL, Uba A, Dukku HU, Wufem BM (2005). An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. J. Ethnopharmacol., 99: 1-4.

Asase A, Oteng-Yeboah AA, Odamtten GT, Simmonds MSJ (2005). Ethnobotanical study of some Ghanaian anti-malarial plants. J. Ethnopharmacol., 99*:* 273-279

Bentley GA, Newton SH, Starr J (1983). Studies on the antinociceptive action of agonist drugs and their interaction with opiod mechanisms. Br. J. Pharmacol., 32: 295-310.

Burkill HM (1995). The useful plants of west tropical Africa, 2nd edition, Royal Botanic Gardens, Kew.

Deradt R, Jougney S, Delevalcee F, Falhout M (1980). Release of prostaglandin E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol., 51: 17-24.

Fortin H, Tomasi S, Jaccard P, Robin V, Boustie J (2001). A prenyloxycoumarin from *Psiadia dentate*. Chem. Pharm. Bull., 49(5): 619-621.

Gene RM, Segura L, Adzet T, Marin E, Inglesias J (1998). *Heterotheca inuloides:* Anti-inflammatory and analgesic effects. J. Ethnopharmacol., 60: 157-162.

Harborne JB (1973). Phytochemical Methods. A Guide to Modern Technique of Plant Analysis. Chapman and Hall Publishers. London, U.K., pp. 55-99.

Herper FN (1976). The West African Herbaria of Isert and Thoning. Bentham-moxin trust in association with Carlsberg foundation, Kew, England, p. 92.

Kim D, Choi S, Kim S, Yun B, Yoo I, Reddy RP, Yoon HS, Kim K (2006). Isoliquiretegenin selectively inhibits H_2 Histamine receptor signaling. Mol. Pharmacol., 70(2): 493-500.

Koster R, Anderson M, DeeBeer EJ (1959). Acetic acid for analgesic screening. Fed. Proc., 18: 412.

Lanhers M, Fleurentin J, Mortier F, Vinche A, Younos C (1992). Antiinflammatory and analgesic effects of an aqueous extract of *Harpagophytum procumbens*. Planta Med., 58: 117-123.

Lee S, Li G, Kim J, Chang H, Jahng Y, Woo M, Song D, Son JD (2003). Two new furanocoumarins from the roots of *Angelica dahurica*. Bull. Korean Chem. Soc., 24(11): 1699-1701.

Levini JD, Lau W, Kwait G, Goetzl EJ (1984). Leukotriene B4 produces hyperalgesia that is dependent on the polymorph-nuclear leucocytes. Science*,* 225: 743-745.

SatoY, He J, Nagai H, Tani T, Akao T (2007). Isoliquiteregenin, one of the anti-spasmodic principles of *Glycyrrhiza ularensis* roots, acts in the lower parts of intestine. Biol. Pham. Bull., 30(1): 145-149.

Silva GL, Lee I, Douglas K (1998). Special problems with extraction of plants. In: Cannel JPR (ed.). Natural Products Isolation. Humana press publishers, New Jersey, pp. 356-358.

Sule MI, Pateh UU, Haruna AK, Garba M, Ahmadu AA, Adamu AK (2003). Plants used in Hausa traditional medicine in Northern Nigeria. J. Trop. Biosci., 3: 17-20.

Takahashi T, Takasuka N, Ligo M, Baba M, Nishino, H, Tsuda H, Okuyama T (2004). Cancer Sci., 95(5): 448-453.

Turner RA (1965). Analgesics. In: Turner, R. A. (ed), Screening Methods in Pharmacology. Academic Press, London, U.K., p. 100.