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Modification of parthenin†

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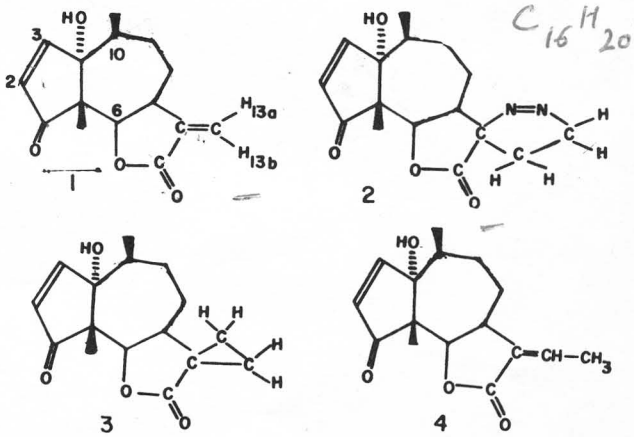
Parthenin, the principal chemical constituent of *Parthenium hysterophorus* weed has been transformed chemically and photochemically with a view to study the structure-activity relationship for allelopathic effects. Chemical transformation gives three products replacing the exocyclic methylene double bond of the lactone ring by a pyrazoline nucleus, a cyclopropane ring and a propenyl group. Photochemical reaction leads to the formation of a hemiacetal and a lactone in place of the cyclopentenone moiety alongwith dehydrated parthenin. Cyclopropyl analogue has been found to be most effective against *Phaseolus aureus* when compared to parthenin.

Parthenium hysterophorus L. (Asteraceae) is an exotic noxious weed accidentally introduced by man through seeds imported alongwith PL 480 wheat grain from USA. Since 1956, it is proliferating extensively. Today about 2 million hectare of land has been declared a national health and agricultural hazard. Its over encroaching tendencies and exclusion of other crops have been found to be due to teletoxic potential (allelopathy) of the plant.

In our earlier studies[1,2] the inhibitory function specificity of the leachates (petroleum ether fraction and methanol fraction) demonstrates the possibility of parthenin being one of the phytotoxins. Hence, attempts were made to transform parthenin, both chemically and photochemically to find out the active moiety responsible for the allelopathic effects in parthenin molecule.

Chemically, parthenin (**1**) was treated with CH_2N_2 to give a colourless solid, m.p. 144°. It was characterized as pyrazoline (**2**) with the help of elemental analysis ($C_{16}H_{20}N_2O_4$) and spectral data. Absorption bands appearing at 3070, 1645, 880 (characteristic of exocyclic double bond $-C(=CH_2)$) in parthenin were found missing and new bands appeared at 1600 (N=N) and 1410 cm^{-1} (C−N vibration) in the IR spectrum of **2**. Similarly, the two doublets appearing at δ 6.33 and 5.72 (J = 2 Hz) of one-proton intensity each in **1** were found to be missing in the PMR spectrum of **2** and instead two distorted triplets appeared at δ4.9 and 5.81 integrating for two protons each. These observations together with positive qualitative test for nitrogen

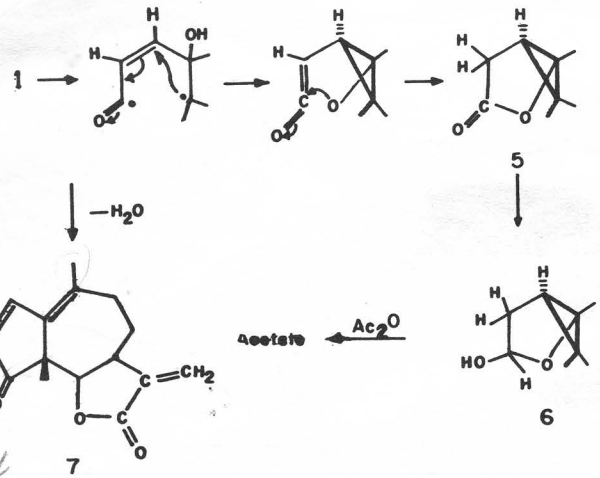


confirmed the formation of pyrazoline nucleus in place of exocyclic methylene double bond.

Pyrazoline (**2**) on heating at 160° for 1 hr gave two compounds **3** and **4**. The nonpolar compound of the two analysed for $C_{16}H_{20}O_4$ and was found to contain a cyclopropane ring (1020 and 845 cm^{-1}; δ0.8-1.1, 4H) while the polar one analysed for $C_{16}H_{20}O_4$ and exhibited a doublet quartet at δ6.8 for 1H (CH_3-C*H*=C<) and a doublet at 1.90 ($J = 7H_z$) for 3 protons ($CH_3-CH=C<$) in the PMR spectrum confirming the formation of a cyclopropyl derivative (**3**) and a propenyl derivative (**4**)

In photochemical reaction, a pure sample of parthenin in methanol was irradiated with a medium pressure Hg lamp for 6 hr. Three compounds **5, 6** and **7** were isolated from the reaction mixture by column chromatography over silica gel.

Compound **5** was obtained as a viscous oil which



on further purification by preparative TLC exhibited a band at 1775 for a saturated γ-lactone in addition to a band at 1760 cm^{-1} (also present in parthenin molecule) for α, β-unsaturated γ-lactone in its IR spectrum. Absence of a band at 3540 cm^{-1} for hydroxyl group indicated the alteration in the cyclopentenone ring. Appearance of a multiplet at δ 2.2 for 2 protons in lieu of two doublets at 6.25 and 7.80 (J = 6 Hz) in the case of parthenin molecule confirmed not only the saturation of the double bond of cyclopentenone ring but the formation of a saturated γ-lactone ring (1775 cm^{-1}). Structure **5** was thus assigned to the lactone which was also proved by fragmentation pattern showing a loss of ($M^+ - CO$) (m/z 262-234) as also a peak at m/z 218) ($M^+ - OC=O$). This product is formed via cyclopentenone cyclopropyl ketene rearrangement[3,4].

The second compound isolated from later fractions of the column was identified as hemiacetal (M^+ 264) with the help of spectral data. Its IR spectrum showed the presence of a hydroxyl group (3460 cm^{-1}) which was confirmed by a signal at δ 3.6 in the PMR spectrum The formation of an acetal was further confirmed by conversion into acetate with acetyl chloride and pyridine. The hemiacetal was assigned structure **6**. Mechanism leading to the formation of **6** has been proposed to involve partitioning of a biradical (a ketene, trapped as lactone) formed by α-fission of parthenin.

The third photoproduct, which crystallized from benzeneethyl acetate, was obtained as a colourless solid, m.p. 120-24°. Its IR spectrum was devoid of a peak at 3540 cm^{-1}. Appearance of the molecular ion at m/z 244 and its fragmentation pattern [m/z 216 (M^+-CO) and 200 (M^+-OCO)] indicated it to be a dehydrated parthenin. A similar product is already reported to be formed by the action of BF_3 on parthenin. Thus, structure **7** was assigned to this photoproduct.

The crude products of chemical and photochemical transformations were examined for their various effects on *Phaseolus aureus* against comparable concentrations of pure samples of parthenin. At a concentration of 80 mg per litre both derivatives (derived after chemical or photochemical transformation) significantly reduced the radicle length especially when compared to that of water treated control or parthenin at the comparable concentration. The plumule length was also reduced significantly; however, compared to parthenin the impact was statistically insignificant (Table 1).

Based on these favourable results, studies were further extended with purified derivatives like pyrazoline, cyclopropyl or propenyl derivatives alongwith parthenin at 0.02% concentration. It is clear from Fig. 1 that per cent germination and seed vigour were hardly affected; however, lengths of radicle and plumule in particular were significantly reduced compared to control. Most effective, however, was propenyl derivative where plumule length was reduced by nearly five times.

Studies conducted on nature plants of *P. aureus* show that only parthenin could reduce the content of total chlorophyll significantly (Table 2). As regards the adverse effect on the cell survival values with

Table 1—Effect of different concentrations of parthenin, pyrolysed parthenin and photo-parthenin on the lengths of radicle and plumule of the seedlings of *Phaseolus aureus* Roxb

Compound	Concn. (mg/litre)	Mean length (cm)	
		Radicle	Plumule
Control	0.0	5.38[a]	10.51[cde]
Parthenin	5	5.36[a]	12.08[cd]
	10	5.27[a]	11.75[cd]
	20	4.97[a]	10.10[de]
	40	4.30[a]	7.86[ef]
	80	4.85[a]	6.40[f]
Pyrolysed parthenin	5	6.94[a]	16.65[a]
	10	5.70[a]	14.18[ab]
	20	5.52[a]	12.24[bcd]
	40	5.18[a]	9.60[de]
	80	3.87[b]	6.44[f]
Photo-parthenin	5	5.34[a]	12.75[bc]
	10	5.16[a]	12.45[bcd]
	20	4.72[a]	9.61[de]
	40	4.85[a]	6.28[f]
	80	3.87[b]	5.79[f]

Seed vigour and seed germination was 100% in all treatments including control

* Mean values having same superscripts do not differ from each other at 5% level of significance applying DMRT (Duncan, 1955).

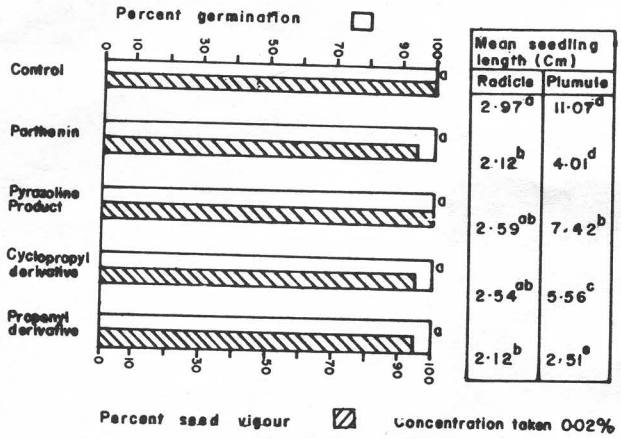


Fig. 1—Effect of parthenin and its chemical transformation and photolytic products on per cent germination, seed vigour and seedling length of *Phaseolus aureus* seeds

Table 2—Effect of parthenin, its pyrazoline product, cyclopropyl derivative and propenyl derivative on per cent chlorophyll, cell survival and water content of *Phaseolus aureus* Roxb. leaves at 0.02%

Treatment	Per cent chlorophyll content w.r.t. control	Per cent cell survival w.r.t. control	Per cent water content w.r.t. control
Control	100[a]	100[a]	100[a]
Parthenin	91.8[b]	77.6[b]	94.9[b]
Pyrazoline poduct of parthenin	101.6[a]	73.3[c]	94.11[b]
Cyclopropyl derivative	99.1[a]	66.3[e]	95.5[b]
Propenyl derivative	98.2[a]	70.2[d]	90.3[c]

Mean values having same superscripts do not differ from each other at 5% level of significance applying DMRT (Duncan, 1955)

respect to control, the maximum impact was observed with cyclopropyl derivative followed by propenyl derivative and least by parthenin. Water content also decreased by these treatments (Table 2). Propenyl derivative was relatively more effective.

It becomes evident that parthenin-caused phytotoxicity could be changed by making structural changes. However, the modified derivatives, besides bringing quantitative changes in some of the responses also lead to qualitative changes in the mode of inhibitory action.

Experimental procedure

All melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer spectrophotometer, PMR spectra on a Varian EM-360L 60 MHz instrument using TMS as internal reference and mass spectra on a JEOL-JMS-D 300 mass spectrometer. Purity of the compounds was checked by TLC on silica gel G plates.

Isolation of parthenin (**1**)

Whole plants of *Parhtenium hysterophorus* collected from Chandigarh were extracted with alcohol after drying under shade and powdering. Parthenin (**1**) was isolated as colourless fine needles, m.p. 165° by rigorous column chromatography over silica gel and characterized with the help of spectral data: IR ($CHCl_3$): 3570, 3540, 3070, 1750, 1710, 1690, 1645, 970, 880 cm^{-1}, PMR ($CDCl_3$): δ 7.80 (d, 1HJ, J = 6Hz, C_2 − H), 6.25 (d, 1H, J = 6Hz, C_3-H), 6.33 (d, 1H, J = 2 Hz, C_{13b} − H), 5.72 (d 1H, J = 2 Hz, C_{13a}-H), 5.02 (d, 1H, J = 7 Hz, C_6-H) 3.96 (bs, 1H, −OH, collapsed on exchange with D_2O) 3.63 (m, 1H, C_7-H), 2.26 (m, 5H, C_8, C_9, and C_{10}-H), 1.30 (s, 3H, C_5-CH_3) 1.14 (d, 3H, J = 7Hz, C_{10}-CH_3).

Reaction of **1** with CH_2N_2

A solution of **1** (1.5 g) in Et_2O (100 ml) was allowed to react with an ethereal solution of CH_2N_2 until the yellow colour persisted for 30 min at room temperature. The product (**2**) was crystallised from ethanol, m.p. 144°; IR ($CHCl_3$): 3560, 1750, 1710, 1600, 1410, 970 cm^{-1}; PMR ($CDCl_3$): δ7.81 (d, 1H, J = 6Hz, C_2-H), 6.25 (d, 1H, J = 6Hz, C_3-H), 5.02 (d, 1H, J = 6Hz, C_6-H), 5.81 (t, 2H, J = 6Hz, N − CH_2), 4.90 (t, J = 6Hz, O = C − C − CH_2 −), 3.61 (m, 1H, C_7-H), 2.20 (m, 5H, C_8, C_9, and C_{10}-H), 1.30 (s, 3H, C_5-CH_3), 1.20 (d, 3H, J = 7Hz, C_{10}-CH_3) ((Found: C, 63.1; H, 6.5; N, 9.2. $C_{16}H_{20}N_2O_4$ requires C, 63.1; H, 6.4; N, 9.2%).

Pyrolysis of **2**

Pyrazoline (**2**) was heated at 160° for 1 hr and the product chromatographed over silica gel to give two compounds **3** and **4**. The nonpolar compound (**3**) of the two had m.p. 125°; IR ($CHCl_3$): 3560, 1740, 1710, 1020, 965, 845 cm^{-1}; PMR ($CDCl_3$): δ7.63 (d, 1H, J = 6Hz, C_2-H), 6.17 (d, 1H, J = 6 Hz, C_3-H), 5.02 (d, 1H, J = 6Hz, C_6-H), 3.5 (m, 1H, C_7-H), 2.24 (m, 5H, C_8, C_9, and C_{10}-H) 1.30 (s, 3H, C_5-CH_3), 1.1 (d, 3H, J = 7Hz, C_{10}-CH_3), 0.80-1.1 (m, 4H, cyclopropyl-H) (Found: C, 69.5; H, 7.3. $C_{16}H_{20}O_4$ requires C, 69.5; H, 7.3%). The polar compound (**4**) showed the following characteristics. PMR ($CDCl_3$): δ7.7 (d, 1H, J = 6 Hz, C_2-H), 6.8 (dq, 1H, J = 2Hz, 7Hz, CH_3 − CH =), 6.23 (d, 1H, J = 6Hz C_3-H), 5.13 (d, 1H J = 6Hz, C_6-H), 3.36 (m, 1H, C_7-H), 2.16 (m, 5H, C_8, C_9, C_{10}-H), 1.9 (d, 3H, J = 7Hz, CH_3 − CH =), 1.33 (s, 3H, C_5-CH_3), 1.1 (d, 3H, J = 7Hz, C_{10} − CH_3) (Found: C, 69.5; H, 7.3. $C_{16}H_{20}O_4$ requires C, 69.5; H, 7.3%).

Photolysis of parthenin

Pure sample of parthenin (**1**; 500 mg) was dissolved in methanol (200 ml) and irradiated with a medium pressure Hg lamp (125 W) through pyrex filter for 6 hr. After irradition for 6 hr, methanol was removed under vacuum and the residue chromatographed over a column of silica gel eluting successively with benzene and benzene-ethyl acetate mixture. Three compounds **5, 6** and **7** were isolated.

Compound **5**, a viscous oil was further purified by preparative TLC; IR (KBr): 1775 and 1760 cm^{-1}; PMR ($CDCl_3$): δ6.35 (d, 1H, J = 2Hz, C_{13}b-H), 5.7 (d, 1H, J = 2Hz, C_{13a}-H), 4.5 (d, 1H, J = 7Hz, C_6-H), 3.6 (m, 1H, C_7-H), 2.9 (m, 3H, C_9 and C_{10}-H), 2.2 (m, 4H, C_8, and C_2-H), 1.3 (s, 3H, C_5-CH_3), 1.05 (d, 3H, J = 7Hz, C_{10}-CH_3), 0.8 (t, 1H, J = 6Hz, C_3-H); MS: m/z 262 (M^+), 234, 218, 205, 180, 164.

Compound **6** was purified by preparative TLC; IR (KBr): 3460 and 1740 cm^{-1}; PMR ($CDCl_3$): δ6.5 (d, 1H, J = 2Hz, C_{13b}-H), 5.7 (d, 1H, J = 2Hz, C_{13a}-H), 4.5 (d, 1H, J = 7Hz, C_6-H), 3.6 (m, 2H, C_7 and C_1-H), 2.9 (m, 3H, C_9 and C_{10}-H), 2.0 (m, 2H, C_8-H), 1.5 (s, 3H, C_5-CH_3), 1.03 (d, 3H, J = 7 Hz, C_{10}-CH_3), 0.8 (t, 1H, J = 7Hz, C_3-H); MS: m/z 264 (M^+).

Compound **7** crystallized from benzene-ethyl acetate to yield colourless crystals, m.p. 120-24°; IR (KBr): 1750 cm^{-1}; PMR ($CDCl_3$): 8.06 (d, 1H, J = 6Hz, C_2-H), 6.45 (d, 1H, J = 2Hz, C_{13b}-H), 6.2 (d, 1H, J = 6 Hz, C_3-H), 5.7 (d, 1H, J = 2 Hz, C_{13a}-H), 4.56 (d, 1H, J = 6 Hz, C_6-H), 3.5 (m, 1H, C_7-H), 2.13 (m, 7H, C_9, C_8-H, C_{10}-CH_3), 1.5 (s, 3H, C_5-CH_3); MS: m/z 244 (M^+), 216, 200.

Bioassay

Bioassay studies involving germination parameters, content of chlorophyll and per cent cell survival were studied in accordance with the method given by Kumari *et al.*[1]. The content of water was studied following the method described by Trease and Evans[6]. The data was statistically analysed using Duncan's multiple Range test[7].

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