

Phytoalexin Production by Species of the Genus *Caragana*

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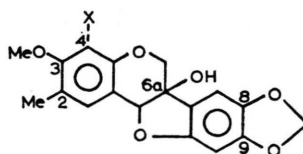
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The fungus-inoculated leaflets of 39 *Caragana* species have been found to variously produce five isoflavonoid phytoalexins including the known pterocarpan derivatives, medicarpin, maackiain, variabilin and pisatin. Small quantities of a previously undescribed C-methylated pterocarpan were obtained from leaflets of *C. acanthophylla*.

The genus *Caragana* (Leguminosae; tribe Astragaleae [1]) contains between 50 and 80 species of spiny, deciduous shrubs and small trees many of which are native to Central Asia [2] and the Far East (China). Several *Caragana* species (e.g. *C. aurantiaca* and *C. arborescens* "Lorbergii") are useful ornamentals whilst others (e.g. *C. jubata* from the Lake Baikal region of Siberia) can be regarded as "botanical curiosities". Although the genus has no current agricultural value, it has been suggested that seeds of *C. arborescens* (Siberian pea shrub) might be used to feed poultry [3]. In a continuing search for new antifungal compounds of plant origin, a representative range of *Caragana* species have recently been examined for their ability to produce isoflavonoid phytoalexins following inoculation of excised leaflets with the fungus, *Helminthosporium carbonum*. The results of this study are outlined below.

Leaf diffusates [4] were treated as described in the preceeding paper and their components then chromatographed (Si gel TLC [5]; CHCl₃ : MeOH, 50 : 1) to afford medicarpin (1) (3-hydroxy-9-methoxypterocarpan) and maackiain (2) (3-hydroxy-8,9-methylenedioxypterocarpan) as a combined zone at approx. *R_F* 0.60. Both compounds were eventually separated by TLC in *n*-pentane : Et₂O : HOAc (75 : 25 : 3, × 3) [6]. As well as 1 and 2, several *Caragana* species also produced small quantities of variabilin (3) (3,9-dimethoxy-6a-hydroxypterocarpan) and pisatin (4) (3-methoxy-6a-hydroxy-8,9-methylenedioxypterocarpan) which co-chromatographed to approx. *R_F* 0.71. All attempts to separate 3 and 4 by Si gel TLC were unsuccessful. Finally, *C. acanthophylla* accumulated a new C-methylated pterocarpan (see below) for which the common name acanthocarpan (5) is proposed. There was no evidence to suggest that leaf diffusates contained other fungitoxic compounds. Pterocarpan 1–5 were not produced by leaflets treated with de-ionised H₂O.



Acanthocarpan (5)

(X indicates alternative position for the Me substituent)

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As shown in Table I, medicarpin and maackiain were present in diffusates from all accessions of the 39 *Caragana* species examined where, as in numerous other papilionate legumes [6–8], they frequently co-occurred in a 1 : 1 (or near 1 : 1) ratio. In contrast, however, certain species, e.g. *C. ambigua* and *C. laeta*, accumulated significantly greater quantities of 1 whilst others, notably *C. gerardiana*, *C. sibirica* and two forms ("Cucullata" and "Lorbergii") of *C. arborescens* produced maackiain as their principal phytoalexin. Samples from different accessions of *C. arborescens* were found to exhibit remarkable variability in terms of their medicarpin/maackiain ratio. The minor pterocarpan, variabilin and pisatin, were of only sporadic occurrence in *Caragana*; apart from *C. jubata* (3) and *C. arborescens* "Pendula" (4), neither compound was present in leaf diffusates at concentrations exceeding 10 µg/ml.

In addition to 1 and 2, diffusates from *C. acanthophylla* also contained small quantities of a hitherto undescribed phytoalexin apparently admixed with traces of variabilin and pisatin. This substance has been provisionally identified [8] as 2-(or 4)-methyl-3-methoxy-6a-hydroxy-8,9-methylenedioxypterocarpan (5) (acanthocarpan; methylpisatin). No induced or constitutive C-methylated pterocarpan have been reported although a 6-methyl substituted isoflavanone (ougenin) is known as a normal heartwood and leaf constituent of the leguminous tree, *Ougeinia dalbergioides* [9, 10] (tribe Desmodieae). The MS of acanthocarpan exhibited prominent frag-

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Table I. Concentration ($\mu\text{g/ml}$) of pterocarpan phytoalexins in diffusates (48 h) from *Helminthosporium carbonum*-inoculated leaflets of *Caragana* species.

Species	Seed Source	MD	MK	V	P	AC	Species	Seed Source	MD	MK	V	P	AC
<i>C. acanthophylla</i> Kom.	T	15	16	TR*	TR*	14	<i>C. praini</i>						
<i>C. altagana</i> Poir.	Gl	14	18	ND	ND	ND	<i>C. K. Schneider</i>	Kh	18	10	—	—	—
<i>C. altaica</i> (Kom.) Pojark	No	46	17	—	—	—	<i>C. pumila</i> Pojark	Mo	13	6	—	—	—
<i>C. ambigua</i> Stocks	Kh	42	23	—	—	—	<i>C. pygmaea</i> (L.) DC.	C	16	12	—	—	—
<i>C. ambigua</i> Stocks	T	29	17	—	—	—	<i>C. sibirica</i> Medic	T	TR	16	4	4	—
<i>C. arborescens</i> Lam.	C	19	21	—	2	—	<i>C. sinica</i> (Buchholz) Rehder	T	18	23	—	—	—
<i>C. arborescens</i> Lam.	S	9	17	ND	ND	ND	<i>C. sophoraefolia</i> Tausch	V	35	41	—	—	—
<i>C. arborescens</i> Lam.	Mo	14	19	TR*	3	—	<i>C. sophoraefolia</i> Tausch	Tu	26	34	—	—	—
<i>C. arborescens</i> Lam. "Albescens"	Mw	20	26	ND	ND	ND	<i>C. spinosa</i> (L.) DC.	Mo	21	25	—	3	—
<i>C. arborescens</i> Lam. "Crassaeaculeata"	N	10	17	ND	ND	ND	<i>C. stenophylla</i> Pojark	K	14	17	—	—	—
<i>C. arborescens</i> Lam. "Cucullata"	L	TR	9	ND	ND	ND	<i>C. tangutica</i> Maxim.	Bn	42	25	—	—	—
<i>C. arborescens</i> Lam. "Cuneifolia"	C	14	17	4	6	—	<i>C. tibetica</i> Kom.	Tu	11	14	—	—	—
<i>C. arborescens</i> Lam. "Lorbergii"	Gu	TR	10	4	5	—	<i>C. tibetica</i> Kom.	V	15	17	—	—	—
<i>C. arborescens</i> Lam. "Nana"	S	12	6	ND	ND	ND	<i>C. tragacanthoides</i> Poir.	Tu	15	18	1	2	—
<i>C. arborescens</i> Lam. "Pendula"	C	22	18	TR*	11	—	<i>C. turkestanica</i> Kom.	D	27	14	3	4	—
<i>C. aurantiaca</i> Koehne	Tu	10	13	—	1	—	<i>C. turkestanica</i> Kom.	S	23	12	2	4	—
<i>C. boissii</i>	Bn	17	9	4	6	—	<i>C. ussuriensis</i> (Regel) Pojark	Ki	11	8	—	—	—
<i>C. K. Schneider</i>	Sm	20	14	4	8	—							
<i>C. boissii</i>	Kh	14	15	4	3	—							
<i>C. K. Schneider</i>	S	20	19	—	4	—							
<i>C. brevispina</i> Royle	T	32	39	2	3	—							
<i>C. chamlagu</i> Lam.	S	17	19	TR*	6	—							
<i>C. conferta</i> Benth.	Gr	14	18	—	3	—							
<i>C. decorticans</i> Hemsl.	W	20	14	—	—	—							
<i>C. decorticans</i> Hemsl.	Gr	2	11	1	2	—							
<i>C. densa</i> Kom.	Kh	8	13	—	—	—							
<i>C. franchetiana</i> Kom.	M	14	16	—	—	—							
<i>C. frutex</i> K. Koch	Kh	24	29	—	—	—							
<i>C. frutex</i> K. Koch "Grandiflora"	Mo	36	25	14	6	—							
<i>C. fruticosa</i> (Pall.) Bess.	T	31	21	—	—	—							
<i>C. gerardiana</i> (Graham) Benth.	Be	TR	36	TR*	4	—							
<i>C. grandiflora</i> DC.	Gl	21	18	—	—	—							
<i>C. jubata</i> (Pall.) Poir.	Mo	36	25	14	6	—							
<i>C. kirghisorum</i> Pojark	T	31	21	—	—	—							
<i>C. laeta</i> Kom.	T	29	9	—	—	—							
<i>C. laeta</i> Kom.	Mo	34	14	—	—	—							
<i>C. macrophylla</i> Lam.	S	30	31	—	4	—							
<i>C. maximowicziana</i> Kom.	Sm	29	10	—	—	—							
<i>C. microphylla</i> Lam.	G	21	29	5	7	—							
<i>C. microphylla</i> Lam. "Megalantha"	Gr	16	23	ND	ND	ND							
<i>C. mollis</i> Bess.	St	21	19	—	—	—							
<i>C. pekinensis</i> Kom.	P	10	14	—	—	—							
<i>C. pleiophylla</i> (Regel) Pojark	Mo	16	20	—	—	—							

Key: MD, medicarpin; MK, maackiain; V, variabilin; P, pisatin; AC, acanthocarpin; ND, not determined; TR, trace; —, not detectable. An asterisk (*) indicates that traces of the phytoalexin (V or P) were detected by MS analysis of pisatin (in the case of V*) or acanthocarpin (V*+P*). Phytoalexin concentrations were determined spectrophotometrically using the following extinction coefficients: 1, $\log \epsilon = 3.90$ at 287 nm [7]; 2, $\log \epsilon = 3.38$ at 310 nm [7]; 3, $\log \epsilon = 3.93$ at 285 nm [15]; 4, $\log \epsilon = 3.86$ at 309 nm [11]. The value for 5 is based on $\log \epsilon$ for 4.

Key to seed sources: Be, Arboretum and Botanic Garden, Natural History Museum, Humboldt University, East Berlin; Bn, Botanic Garden, Berlin-Dahlem Botanical Museum, West Berlin; C, Botanic Garden, University of Cluj-Napoca, Roumania; D, Botanic Garden, USSR, Academy of Sciences, Dushanbe, Tadzhik SSR, USSR; G, Botanic Garden, Gies-sen, West Germany; Gl, National Botanic Gardens, Glasne- vin, Dublin, Eire; Gr, Botanic Garden, Ernst-Moritz-Arndt University, Greifswald, East Germany; Gu, University Ar- boretum, University of Guelph, Ontario, Canada; K, Botanic Garden, Institute of Botany, Kaunas, Lithuanian, SSR, USSR; Kh, Botanic Garden, Khorog, Tadzhik SSR, USSR; Ki, Botanic Garden, USSR Academy of Sciences, Kiev, Ukrainian SSR, USSR; L, V. L. Komarov Botanic Institute, Leningrad, USSR; M, Botanic Garden, USSR Academy of Sciences, Minsk, Belorussian SSR, USSR; Mo, Botanic Garden, USSR Academy of Sciences, Moscow, USSR; Mw, K. A. Timirjasevi Agricultural Academy, Moscow, USSR; N, Botanic Garden, Smith College, Northampton, Massachu- setts, USA; No, Botanic Garden, Novosibirsk, USSR; P, Botanic Garden, Institute of Botany, Chinese Academy of Sciences, Peking, China; S, Mlyňany Arboretum, Slepčany, Czechoslovakia; St, Botanic Garden, Stavropol', USSR; Sm, Botanic Garden, Stockholm, Sweden; T, Botanic Garden, USSR Academy of Sciences, Tashkent, Uzbek SSR, USSR; Tu, Botanic Garden, University of Turku, Finland; V, Bo- tanic Garden, Vacratot, Hungary; W, Botanic Garden, Agricultural University, Wageningen, The Netherlands.

ments at m/e (rel.int.) 328 ($C_{18}H_{16}O_6$; M^+ ; 100), 314 (expected M^+ for pisatin; 11), 313 ($M^+ - Me$; 11), 311 (9), 310 ($M^+ - H_2O$; 42) and 300 (expected M^+ for variabilin; 28). In EtOH **5** gave UV maxima (nm) at 210, 238 sh, 280 sh, 286 sh and 309 although it is probable that the inflections at 280 and 286 nm reflect the presence of **3** and/or **4** rather than a spectral characteristic of acanthocarpan. In fact, the above EtOH spectrum could be almost exactly reproduced by adding small quantities of pisatin to a solution of 2-hydroxy-3-methoxy-8,9-methylenedioxypterocarpan (**6**), a compound which normally exhibits only a single UV (EtOH) maximum at 308 nm. The neutral spectrum of **5** was unaffected by NaOH but with conc. HCl (d. 1.18, 5 drops) acanthocarpan underwent rapid 6a/11a dehydration [11] to give 2-(or 4)-methyl-3-methoxy-8,9-methylenedioxypterocarp-6a-ene which exhibited intense UV maxima at 337 and 354 nm. Further studies are now in progress to determine if the methyl substituent of **5** is located at C-4 or at the more biogenetically acceptable C-2 position. In tests against the mycelial growth of *H. carbonum*, **5** had antifungal activity (ED_{50} approx. 50 $\mu g/ml$) similar to that of pisatin (ED_{50} approx. 40 $\mu g/ml$). Compounds **1** [6], **2** [6] and **3** (ED_{50} approx. 20 $\mu g/ml$ [12]) are considerably more antifungal.

The tribe Astragaleae is composed of 13 genera [1] including *Astragalus*, an immense assemblage of

almost 2000 species. Surveys currently being undertaken in this laboratory suggest that in terms of phytoalexin biosynthesis, the Astragaleae may be divided into two sections with pterocarpan-producing genera such as *Caragana* (**1–5**), *Calophaca* (**1, 2**) [8] and *Halimodendron* (**1, 2**) [8] on the one hand and isoflavan accumulators (*e.g.* *Glycyrrhiza* [13] and *Oxytropis* [8]) on the other. Not surprisingly in view of its size, *Astragalus* appears to occupy an intermediate position having species which produce both pterocarpan (**1, 2**) and isoflavan phytoalexins [8]. Finally, it is noteworthy that whereas **1** and **2** are exceptionally prevalent as legume phytoalexins, both variabilin and pisatin appear to be comparatively rare. Indeed, these latter pterocarpanes occur principally in genera belonging to the tribes Viciae (*Lathyrus*; *Lens*; and *Pisum*), Astragaleae (*Caragana*) and Thermopsidae [14] (*Baptisia* and *Thermopsis*); all three tribes are closely related and, apart from occupying only a small section of the Papilionoideae [14], form a phyletic line leading to the more primitive Sophoreae and Swartzieae where **3** and **4** have also been occasionally detected [8].

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