Phytoalexin Production by Species of the Genus *Caragana*

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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]; $CHCl_3$: 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 : Et_2O : HOAc (75 : 25 : 3, $\times 3$) [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. Pterocarpans **1** – **5** were not produced by leaflets treated with deionised H_2O.

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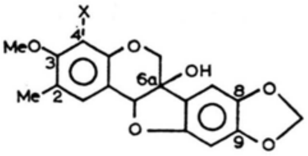

Acanthocarpan (**5**)
(X indicates alternative position for the Me substituent)

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 pterocarpans, 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 pterocarpans 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-

Table I. Concentration (μg/ml) of pterocarpan phytoalexins in diffusates (48 h) from *Helminthosporium carbonum*-inoculated leaflets of *Caragana* species.

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

Key: MD, medicarpin; MK, maackiain; V, variabilin; P, pisatin; AC, acanthocarpan; 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 acanthocarpan (V*+P*). Phytoalexin concentrations were determined spectrophotometrically using the following extinction coefficients: **1**, $\log \varepsilon = 3.90$ at 287 nm [7]; **2**, $\log \varepsilon = 3.38$ at 310 nm [7]; **3**, $\log \varepsilon = 3.93$ at 285 nm [15]; **4**, $\log \varepsilon = 3.86$ at 309 nm [11]. The value for **5** is based on $\log \varepsilon$ 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, Giessen, West Germany; Gl, National Botanic Gardens, Glasnevin, Dublin, Eire; Gr, Botanic Garden, Ernst-Moritz-Arndt University, Greifswald, East Germany; Gu, University Arboretum, 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, Ukranian 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, Massachusetts, 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, Uzbeck SSR, USSR; Tu, Botanic Garden, University of Turku, Finland; V, Botanic 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 μg/ml) similar to that of pisatin (ED_{50} approx. 40 μg/ml). Compounds **1** [6], **2** [6] and **3** (ED_{50} approx. 20 μg/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 pterocarpans occur principally in genera belonging to the tribes Vicieae *(Lathyrus; Lens;* and *Pisum)*, Astragaleae *(Caragana)* and Thermopsideae [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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[1] J. Hutchinson, The Genera of Flowering Plants, **Vol. 1,** 456, Clarendon Press, Oxford 1964.
[2] W. J. Bean, Trees and Shrubs Hardy in the British Isles **1,** 490, John Murray Ltd., London 1970.
[3] L. D. Hills, Ecologist **6,** 343 (1976).
[4] J. L. Ingham and R. L. Millar, Nature **242,** 125 (1973).
[5] J. L. Ingham, Z. Naturforsch. **31 c,** 504 (1976).
[6] J. L. Ingham, Phytopathol. Z. **87,** 353 (1976).
[7] J. L. Ingham, Biochem. Syst. Ecol. **6,** 217 (1978).
[8] J. L. Ingham, unpublished data.
[9] S. Balakrishna, J. D. Ramanathan, T. R. Seshadri, and B. Venkataramani, Proc. Royal Soc. **268 A,** 1 (1962).
[10] V. K. Ahluwalia, G. P. Sachdev, and T. R. Seshadri, Indian J. Chem. **4,** 250 (1966).
[11] D. R. Perrin and W. Bottomley, J. Amer. Chem. Soc. **84,** 1919 (1962).
[12] D. J. Robeson, Ph. D. thesis, University of Reading, U. K. 1978.
[13] J. L. Ingham, Phytochemistry **16,** 1457 (1977).
[14] E. A. Bell, J. A. Lackey, and R. M. Polhill, Biochem. Syst. Ecol. **6,** 201 (1978).
[15] C. W. L. Bevan, A. J. Birch, B. Moore, and S. K. Mukerjee, J. Chem. Soc. **1964,** 5991.