beans) have been used in Indian rituals because of their purported hallucinogenic effect [1–3]. This has led to their utilisation in the modern drug scene [4, 5]. *Previous work.* There is only one report concerning the actual isolation of an alkaloid (cytisine) from *S. secundiflora* (seeds) [6]. Another report deals with the absence of cytisine in the leaves [7].

Present work. Powdered seeds (1 kg) were extracted with EtOH in a large Waring blender. A crude alkaloid fraction was obtained by working up the extract in the usual fashion. The entire crude alkaloid fraction was subjected to PLC on Si gel with $CHCl_3$–MeOH–28% NH_4OH (100 : 10 : 1) to yield 3 major bands, *A–C*.

Band A was processed to yield 1·51 g (0·15%) of cytisine; perchlorate, mp 294° (lit. [8] 296°) and picrate, mp 276–277° (lit. [9] 276–279°). *Band B* was rechromatographed (PLC) on Si gel with EtOAc–MeOH–28% NH_4OH (17:2:1) to give 345 mg (0·03%) of sparteine; sulfate, mp 263–264° (lit. [10] 264–265°) and methiodide, mp 238–239° (lit. [10] 237–238°). *Band C* was rechromatographed (PLC) on Si gel with $CHCl_3$–MeOH (5:1) to give 459 mg (0·04%) of *N*-methylcytisine; perchlorate, mp 281–282° (lit. [11] 282°) and picrate, mp 229–230° (lit. [12] 230°). All three alkaloids were identified by comparing the IR and NMR spectra with those of authentic samples and co-chromatography (TLC 3 solvents) in addition to mp. TLC of the crude alkaloid fractions from leaf and pod material revealed the presence of the same 3 major alkaloids that were isolated from the seeds.

Significance. The presence of cytisine in *S. secundiflora* seeds has often been cited as the basis for the past and present use of this psychotropic plant [1–4]. The present work verifies the presence of cytisine and suggests that 2 other major alkaloids in the seeds may contribute to the hallucinogenic activity.

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REFERENCES

1. Schultes, R. E. (1963) *Psychedelic Rev.* **1**, 145.
2. Schultes, R. E. (1969) *Science* **163**, 245.
3. Farnsworth, N. R. (1972) *J. Psychedelic Drugs* **5**, 67.
4. Superweed, M. J. (1970) *Herbal Highs*, p. 6. Stone Kingdom Syndicate, San Francisco.
5. Lehnert, E. (Chief Naturalist, Amistad Recreation Area, Del Rio, Texas), personal communication, September 19, 1974.
6. Plugge, P. C. (1895) *Arch. Pharm.* **233**, 294.
7. Carsmada, R. S. M. (1948) *Mon. Farm Terap.* **54**, 281; (1949) *Chem. Abstr.* **43**, 1910a.
8. Briggs, L. H. and Russell, W. E. (1942) *J. Chem. Soc.* **1942**, 507.
9. Keller, W. J. and Cole, F. R. (1969) *Lloydia* **32**, 498.
10. Couch, J. F. (1937) *J. Am. Chem. Soc.* **59**, 1469.
11. Marion, L. and Cockburn, W. F. (1948) *J. Am. Chem. Soc.* **70**, 3472.
12. Briggs, L. H. and Taylor, W. S. (1938) *J. Chem. Soc.* **1938**, 1206.

Phytochemistry, 1975, Vol. 14, pp. 2306–2308. Pergamon Press. Printed in England.

NITRO COMPOUNDS IN *ASTRAGALUS* SPECIES

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Key Word Index—*Astragalus*; Leguminosae; nitro compounds; miserotoxin; cibarian; karakin; 3-nitro-1-propanol; 3-nitropropionic acid.

All numbered voucher specimens of *Astragalus* spp. described here are in the Intermountain Herbarium, Utah State University, Logan, Utah, U.S.A. 84322. *Plant, A. canadensis* var. brevidens (Gand.) Barneby, No. 138960; *source*, Custer Co., Idaho, U.S.A.; *part examined*, aerial portion. *Plant, A. convallarius* Greene, No. 138958; *source*, Oneida Co., Idaho, U.S.A.; *part examined*, aerial

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Table 1. High pressure liquid chromatography of 10 *Astragalus* species for nitro compounds

Astragalus species	Nitro Compounds present (% of total)*† Miserotoxin (0·04)	Cibarian (0·09)	Karakin (0·23)	Hiptagin (0·34)	3-NPOH* (0·46)	3-NPA† (0·63)
A. convallarius var. *convallarius*	25	43	8	2	19	3
A. diversifolius	64	14	6		16	
A. tetrapterus	96		2		2	
A. pterocarpus	47	38	11		4	
A. falcatus	23	26	20	10	12	9
A. canadensis var. *brevidens*	3	31	35	5	14	12
A. emoryanus var. *emoryanus*	22	35	11	12	20	
A. toanus	23	15	15		37	10
A. siliquosus	68	17			15	
A. galegiformis	32	18	15		35	

* 3-NPOH = 3-Nitro-1-propanol. 3-NPA = 3-Nitropropionic acid.
† R_f values TLC in 1:1 $CHCl_3$–Me_2CO on SiO_2 in brackets. In addition an unknown R_f 0·18 was present in *A. canadensis*, *A. falcatus* and *A. emoryanus*.

portion. *Plant*, *A. diversifolius* Gray, No. 135836; *source*, Custer Co., Idaho, U.S.A.; *part examined*, aerial portion. *Plant*, *A. emoryanus* (Rydb.) Cory, No. 138947; *source*, Lincoln, Co., New Mexico, U.S.A.; *part examined*, aerial portion. *Plant*, *A. falcatus* Lam., No. 138935; *source*, Cache Co., Utah, U.S.A. Plants grown from seed collected in Georgia, U.S.S.R. P.I. No. 314062. *Use.* Legume for seeding on western range; *part examined*, aerial portion. *Plant*, *A. galegiformis* L., No. 138936; *source*, Cache Co., Utah, U.S.A. Plants grown from seed collected near Pre Mt., North Caucasus (Isprannaya village), U.S.S.R. P.I. No. 314062; The species is being evaluated for possible introduction on rangeland in the western U.S.A.; *part examined*, aerial portion. *Plant*, *A. pterocarpus* Wats., No. 135052; *source*, Lander Co., Nevada, U.S.A.; *part examined*, aerial portion. *Plant*, *A. siliquosus* Boiss., No. 138941; *Source*, Cache Co., Utah. U.S.A. Plants grown from seed collected in Eastern Malayer, Iran, P.I. No. 330696. The species is being evaluated for possible introduction on rangeland in the western U.S.A.; *part examined*, aerial portion. *Plant*, *A. tetrapterus* Gray, No. 138959; *source*. Lincoln Co., Nevada, U.S.A.; *part examined*, leaves. *Plant*, *A. toanus* Jones, No. 138586; *source*. Elko Co., Nevada, U.S.A.; *plant part examined*. aerial portion. On all above previous work refers to nitro analysis [1–3] and toxicity [2,3].

Previous investigations established the occurrence in *Astragalus* spp. of the nitro compounds miserotoxin, cibarian, karakin, hiptagin, 3-nitro-1-propanol (3-NPOH), and 3-nitropropionic acid (3-NPA) [4–8]. Upon acid hydrolysis, the nitro side chain of miserotoxin forms 3-NPOH, whereas the nitro catabolite of cibarian, karakin, and hiptagin becomes 3-NPA. Since 3-NPOH is more toxic than 3-NPA (mg NO_2/kg of body wt) to chicks, sheep, and cattle, [2] we concluded that the more toxic *Astragalus* spp. synthesized primarily miserotoxin or 3-NPOH, whereas the less toxic species synthesized predominantly 3-NPA or nitro compounds that catabolized to 3-NPA in the digestive tract of ruminants.

We examined 10 species of *Astragalus* for type and concentration of organic nitro compounds by the Griess-Ilosvay TLC spray method and the high pressure liquid chromatography method previously described [7]. The distribution of miserotoxin, cibarian, karakin, hiptagin, 3-NPOH, and 3-NPA isolated by high pressure liquid chromatography are given in Table 1. Relative amounts of each nitro compound determined by TLC were roughly in accord with these results.

Biological significance. A. convallarius, *A. diversifolius*, *A. pterocarpus*, *A. siliquosus*, *A. tetrapterus* and *A. toanus* are more toxic per milligram of contained NO_2 to chicks, domestic ruminants, (or both) than *A. falcatus* and *A. canadensis* [2]. *A. emoryanus* appears intermediate in toxicity. *A. galegiformis*, which rarely contained more than 2 mg NO_2/g plant, was nontoxic in our tests [3]. These data support the hypothesis previously

advanced that the more toxic nitro-bearing *Astragalus* spp. are those in which miserotoxin and 3-NPOH account for nearly half or more of the total nitro content [2].

REFERENCES

1. Williams, M. C. and Parker, R. (1974) *Weed Sci.* **22**, 259.
2. Williams, M. C. and James, L. F. (1975) *J. Range Manage.* **28**, 260.
3. Williams, M. C., James, L. F. and Bleak, A. T. (1975) *J. Range Manage.* In press.
4. Stermitz, F. R., Norris, F. A., and Williams, M. C. (1969) *J. Am. Chem. Soc.* **91**, 4599.
5. Stermitz, F. R., Lowry, W. T., Norris, F. A., Buckeridge, F. A., and Williams, M. C. (1972) *Phytochemistry* **11**, 1117.
6. Stermitz, F. R. Lowry, W. T., Ubben, E., and Sharifi, I. (1972) *Phytochemistry* **11**, 3525.
7. Harlow, M. C., Stermitz, F. R., and Thomas, R. D. (1975) *Phytochemistry* **14**, 1421.
8. Stermitz, F. R., Hnatyszyn, O., Bandoni, A. L., Rondina, R. V. D., and Coussio, J. D. (1975) *Phytochemistry* **14**, 1341.

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CRATAEGOLSÄURE UND STEROIDGLUKOSIDE AUS BLÜTENKNOSPEN VON *SYZYGIUM AROMATICUM*

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Key Word Index—*Syzygium aromaticum*; Myrtaceae; Cloves; 2α-hydroxyoleanolic acid; sitosterol; stigmasterol; campesterol.

Bekannt ist, daß Nelken—die schon vollständig entwickelten, aber noch nicht aufgeblühten Blütenknospen des Gewürznelkenbaumes (*Syzygium aromaticum* L.)—Oleanolsäure (2%) enthalten [1]. Äther löst aus Nelken, wie das DC zeigt, noch zwei weitere Verbindungen heraus, die mit Vanillin–Schwefelsäure gleichfalls blaue bis blauviolette Farbflecken ergeben. Beide Verbindungen verhalten sich polarer als Oleanolsäure ($R_f = 0{\cdot}32$). Durch Säulenchromatographie an SiO_2 [Petrol–Et_2O–MeOH (12:7:1)] werden alle drei Substanzen präparativ getrennt. Die Substanz mit dem $R_f = 0{\cdot}21$ schmilzt, umkristallisiert aus MeOH, bei 265–267°. Nach dem Methylieren ist sie lt. IR, NMR und MS mit 2α-Hydroxyoleanolsäuremethylester (Crataegolsäure-methylester, Fp 217–219°) identisch [2]. Ihre Menge in den Nelken beträgt 0·006%. In den Blättern von *Psidium guaijava L.* [3] und in den Blüten von *Eugenia jambolana* [4], beides Myrtaceen, ist Crataegolsäure bereits nachgewiesen worden.

Die Substanz mit dem $R_f = 0{\cdot}1$ schmilzt nach Umkristallisieren aus Dioxan bei 290°. Ihr Verhalten bei den Farb-reaktionen nach Liebermann-Burchard [5] und Molisch–Udránzki [6] wies auf Steroidglykoside hin. Nach saurer Hydrolyse und Abtrennen der Aglyka werden durch GC und MS Sitosterol, Stigmasterol und Campesterol nachgewiesen. Der Zuckeranteil wird als Glucose identifiziert. Nach den von uns ermittelten Molekulargewichten liegt jedes der drei Steroide an eine Molekel Glukose gebunden vor. Das ß-D-Glukosid von Sitosterol stellt den Hauptanteil dar.

Angaben zum Vorkommen von Steroidglukosiden in Myrtaceen konnten wir in der Literatur nicht ermitteln. Freie Steroide werden beschrieben [7–9].

EXPERIMENTELLES

2α-Hydroxyoleanolsäuremethylester (*Crataegolsäuremethylester*). NMR ($CDCl_3$): δ 5·26 (*t*, 1H, C-12); 3·59 (*s*, 3H, –OMe C-17); 2·97 (*d*, 1H, *J* 9·8 Hz, C-3); 2·65 (*s*, 2H, 2 OH, C-2, C-3); 1·11; 0·98; 0·96; 0·90 (2); 0·82; 0·71 (7 × –Me) ppm MS (70 eV): *m/e* 486 M^+, 468, 450, 426, 408, 391, 343, 262, 249, 223, 203 (Basispeak), 189, 187, 133. IR (KBr) cm^{-1}: 3400 (OH), 1725 (C=O), 1250 (C–O–C).

Steroidglukoside. MS (70 eV): *m/e* 576 M^+ (Sitosterol-glukosid), 574 M^+ (Stigmasterolglukosid), 562 M^+ (Campesterol-glukosid. Molekülpeaks der Aglyka: 414 (Sitosterol), 412 (Stigmasterol), 400 (Campestrol, 73, 60 (typ. Fragmente für Glukose).