Phytochemistry, Vol. 27, No. 3, pp. 737–741, 1988.
Printed in Great Britain.

0031–9422/88 $3.00 + 0.00

ECDYSTEROIDS FROM *LEUZEA CARTHAMOIDES*

J.-P. GIRAULT, R. LAFONT,*† E. VARGA,‡ ZS. HAJDU,‡ I. HERKE‡ and K. SZENDREI‡

Université René Descartes, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, C.N.R.S. U.A. 400, 45 rue des Saints-Pères, F-75270 Paris Cedex 06, France;† Ecole Normale Supérieure, Laboratoire de Biochimie et Physiologie du Développement, C.N.R.S. U.A. 686, 46 rue d'Ulm, F-75230 Paris Cedex 05, France;‡ Department of Pharmacognosy, University Medical School, P.O. Box 121, H-6701 Szeged, Hungary

(*Received* 5 *June* 1987)

Key Word Index—*Leuzea carthamoides*; Compositae; ecdysteroids carthamosterone; 5-deoxykaladasterone.

Abstract—20-Hydroxyecdysone, 24(28)-dehydromakisterone A, makisterone C, polypodine B, ajugasterone C, together with the two new phytoecdysteroids carthamosterone and 5-deoxykaladasterone, were isolated from the roots of *Leuzea carthamoides*. The herb yielded 20-hydroxyecdysone, 24(28)-dehydromakisterone A, makisterone C and carthamosterone.

INTRODUCTION

The multiple physiological and ecological functions established or postulated for ecdysteroids in living organisms [1, 2] make these compounds attractive targets for continued research. The constantly increasing number and structural diversity of known ecdysteroids, especially from plant sources [1] is steadily increasing due to efforts for new and/or more promising sources for such compounds.

Leuzea carthamoides DC. (syn. *Rhaponticum carthamoides* Willd./Iljin), an endemic species in Siberia is extensively being used in traditional and officinal medicine as a natural roborant and stimulant [3]. Earlier research has established that the plant contains 20-hydroxyecdysone (**1**) in substantial amounts and this compound has been considered to bear at least partially the physiological activities of the extracts. The presence of inokosterone (**8**), a related ecdysteroid has also been reported [4].

The plant was introduced into Hungary some 15 years ago and is being cultivated on a large scale. Analytical studies on concentrates using various chromatographic techniques revealed the presence of a series of ecdysteroid-type compounds both in the herb and in the radix. The present paper reports on the isolation and characterization of seven ecdysteroids; five of them are already known compounds, one is a new natural product (**6**) and the trivial name 'carthamosterone' is proposed for it. The last one (**7**) might represent an artifact arising from ajugasterone C (**5**) by dehydration during the purification process.

RESULTS

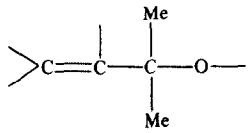
Ecdysteroids were isolated as described in the Experimental. Among the isolated compounds, several of them were already known ecdysteroids (20-hydroxyecdysone, polypodine B, makisterone C, ajugasterone C and 24(28)-dehydromakisterone A), and their identification are given in the Experimental. We report here the establishment of the structures of two new compounds, carthamosterone and 5-deoxykaladasterone.

Identification of carthamosterone (**6**)

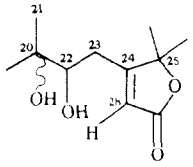
This compound was obtained as an amorphous solid. Its UV spectrum showed two maxima, the larger one at λ_{max} 210 nm, the second one at 242 nm. This was indicative of the presence of an additional absorbing functional group which is independent of the unsaturated ketone on ring B. The IR-FT spectrum gave an additional band (with regard to 20-hydroxyecdysone) in the carbonyl area at ν_{max} 1745 cm^{-1} which is in agreement with the carbonyl signal of an α,β ethylenic γ-lactone [5]. The mass spectrum (CI/D) gave a M_r of 518 indicating the possible formula ($C_{29}H_{42}O_8$). Fragments at *m/z* 363 and 345 were identical with those in the spectrum of 20-hydroxyecdysone and indicate that the differences probably concern the side-chain. Fragments at *m/z* 393 and 375 might arise from cleavage between C-22 and C-23, but these fragments were not found in the EI mass spectrum. The ^1H NMR spectrum showed no important difference with regard to 20-hydroxyecdysone concerning the signals of the ABCD rings, but strong modifications were noted for the signals of the side-chain. The 22-H signal was shifted downfield and coupled to the two strongly downfield shifted 23-$H_{a,b}$ signals (δ2.57, 23-H_a and 2.28, 23-H_b); such chemical shifts are typical of allylic protons. These two protons were weakly coupled to a new ethylenic signal at δ5.92 and the 24-$H_{a,b}$ signals had disappeared. The 26- and 27-methyl signals were also shifted downfield (δ1.48), and this chemical shift data is in accordance with partial structure **A**. The IR spectrum is in agreement with an α,β ethylenic γ-lactone, and from all the above data the two structures **B** or **C** are possible for the side-chain.

The chemical shift of the ethylenic proton (δ5.92, H-28 was only in agreement with a proton in an α position of a α,β-unsaturated carbonyl system [6] and this allows us to eliminate the structure **C** where the H-28 is in a β position.

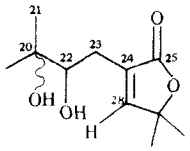
*Author to whom correspondence should be addressed.



A



B



C

Identification of 5-deoxykaladasterone (7)

Compound (7) was identified as 25-deoxy-9(11)-dehydro-20-hydroxyecdysone based on the following points. Its UV spectrum in EtOH showed a strong maximum at λ_{max} 298 nm, (and a minor one at 235 nm); such a maximum value is characteristic of a conjugated dienone and was previously found for kaladasterone [7, 8]. The mass spectrum (CI/D) gave a M_r of 462. The presence of fragments at *m/z* 361, 343 (361-18) and 325 (345-18) is characteristic of an ecdysteroid bearing a 20,22 diol and no 25-OH and an extra double-bond somewhere on the steroid nucleus (ponasterone A, 25-deoxy-20-hydroxyecdysone, has a M_r of 464 and gives nuclear fragments at *m/z* 363, 345 and 327). ^{1}H NMR spectrometry (1D and 2D-COSY experiments) allowed us to assign the 25-deoxy structure based on the chemical shifts and the doublet pattern of 26-Me and 27-Me. The $\Delta^{9(11)}$ structure was established from the disappearance of the 9-H signal and the appearance of a new ethylenic proton signal at δ6.3 coupled with three signals 2.72 ($J=2$ Hz), 2.42 ($J=6.5$ Hz) and 7-H signal by a small long-range coupling ($^5J<2$ Hz, observed with a 2D-COSY experiment). The two signals at δ2.72 (*br d*) and 2.42 (*br dd*) presented a *gem* coupling constant of-18 Hz and can be assigned to 12-H$_{ax}$ and 12-H$_{eq}$ respectively. Small long-range couplings for these two protons with the 7-H signal and one for the 12-H$_{ax}$ with the 18-Me [6] were observed with the 2D-COSY experiment. The observed downfield shifts of the 19-Me, 1-H$_{ax}$, 1-H$_{eq}$ and the small upfield shifts of 2-H$_{ax}$, 3-H$_{eq}$ and 7-H are in accordance with a $\Delta^{9(11)}$ structure.

DISCUSSION

The family Asteraceae was already known to contain ecdysteroids [1]. Besides 20-hydroxyecdysone, which is the major component [9–11], several other ecdysteroids had previously been isolated: 24(28)-dehydromakisterone A (**2**) [12], integristerones A and B [11, 13, 14], sogdysterone [15] and viticosterone E [16]. A previous report [4] from the same species as used in the present paper gave evidence for 20-hydroxyecdysone (**1**) and inokosterone (**8**). However the latter compound was not found in the present study. Instead of it, we have isolated compound **2** and several other ecdysteroids, not previously reported to occur in Asteraceae. Two of them, **6** and **7**, are new compounds.

Carthamosterone (**6**) represents an unusual ecdysteroid. While the presence of a saturated (or an unsaturated) lactone on the side chain occurs in several ecdysteroids, it always involves the formation of a carboxylic function at C-26 (or 27) [1, 2]. In the present case, there is an acidic function at C-29. Such a structure was found for the fungal hormone antheridiol [17], a compound which shares some resemblance with ecdysteroids and would be formed from isofucosterol (a C-29 sterol bearing a 24(28)-ene).

5-Deoxykaladasterone (**7**) is also a new compound. Its co-occurrence with ajugasterone C (**5**) raises the question: is compound (**7**) really present in the plant or does it result from dehydration of **5** during processing? A similar question was previously raised concerning the compounds muristerone A/kaladasterone [8] which only differ from the present compounds by the presence of an additional 5β-OH group, and in that case the authors opted for the natural presence of kaladasterone. This problem is still being investigated in the case of *Leuzea* extracts (Szendrei, K., unpublished work).

Finally, we wish to mention here how powerful are two-dimensional proton NMR techniques for structure elucidation of ecdysteroids isolated in small amounts [18–22]. Such techniques are becoming widely used in the field of natural product chemistry.

EXPERIMENTAL

General procedures. Sample processing included methanolic extraction and several chromatographic steps comprising CC and prep. TLC.

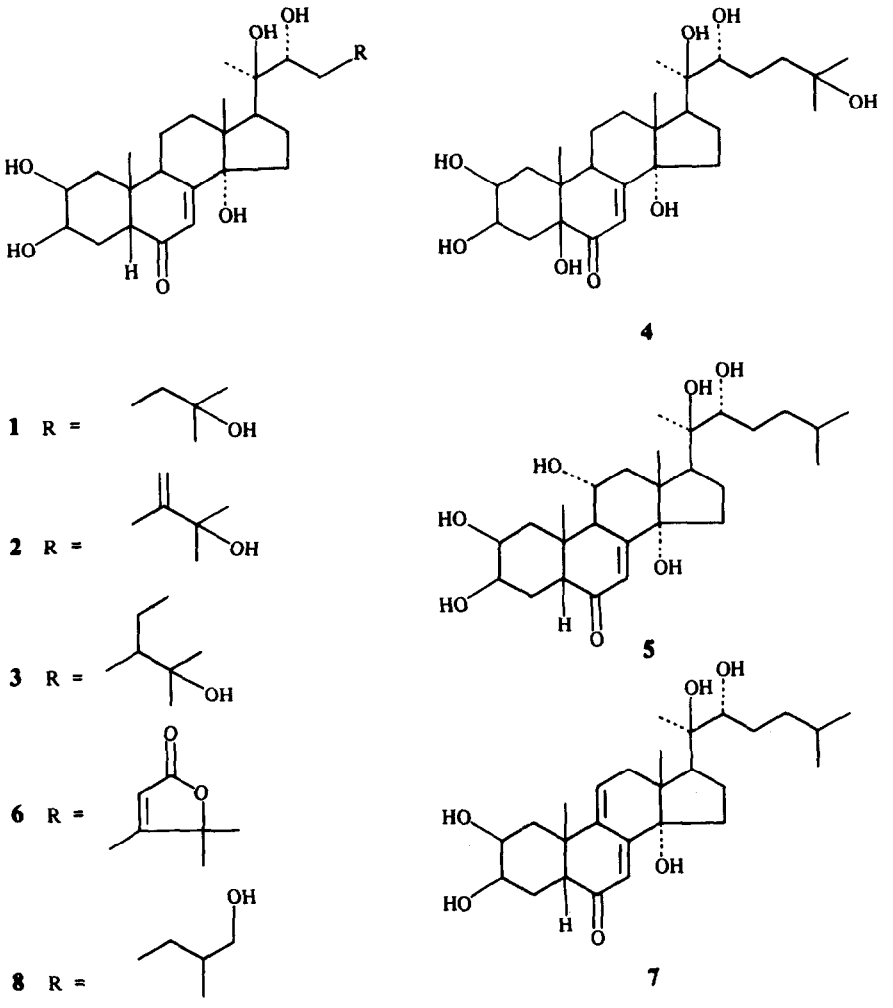
The purity of each isolated fraction was assessed by semi-prep. NP-HPLC and analytical RP-HPLC (Table I) using a Waters system [23, 24].

Pure ecdysteroids were analysed for UV absorbance and for MS (direct inlet probe in the EI mode or the CI/D mode with NH_3 as the reagent gas) [25]. IR-FT spectrometry was performed with a Nicolet 5DX apparatus.

For obtaining ^{1}H NMR spectra (with a Bruker WM250 apparatus), compounds were first lyophilized in D_2O (99.95%) before being dissolved in CD_3OD, reference $TSPd_4$ [sodium 3-(trimethylsilyl)-1-propionate-2,2,3,3 d_4]. The ^{1}H NMR spectra were recorded using standard Fourier transform techniques at 20°. Analyses of spectra was as previously described [22] by homonuclear 2D correlation spectroscopy (2D-COSY) [26] and with its relayed version [27, 28].

Isolation of the ecdysteroids. The plant material originated from a large-scale cultivation at the University of Horticulture Budapest. Herb and root were collected at various developmental stages during the year for qualitative chromatographic analysis. The large samples were collected in May (herb) and in September (roots).

Roots. The dried pulverized plant material (5.5 kg) was extracted at room temp with 70 l MeOH. The soln was evapd under red. pres. and the residue was dissolved in 50% aq. MeOH. The filtered soln was extracted with C_6H_6 (12 × 1 l). The aq. phase was diluted with 1 vol. Me_2CO and the ppt. was filtered off. The remaining soln was concd *in vacuo* and the residue (111 g) was purified on an Al_2O_3 column (500 g Alumina Brockman II; elution with CH_2Cl_2–MeOH 9:1; 300 ml fractions). The fractions containing ecdysteroids (monitoring by TLC) were combined and the mixture (12.45 g) was fractionated on a silica column (300 g silica Merck; elution with a CH_2Cl_2–EtOH gradient; 100 ml fractions).



Fractions eluted with CH_2Cl_2–EtOH (96:4 and 90:10) contained three ecdysteroids. They were separated by prep. alumina TLC in EtOAc–MeOH–NH_3 (85:10:5). Elution of the three bands with MeOH yielded 1.8 mg 24(28)-dehydromakisterone A (**2**), 4.2 mg makisterone C (**3**) and 1 mg carthamosterone (**6**). Fractions eluted with CH_2Cl_2–EtOH (90:10), which contained some material formed in the previous fractions, together with some 20-hydroxyecdysone, yielded two further polar ecdysteroids. The residue of these combined fractions (1.48 g) was separated on a polyamide column (45 g Polyamid Woelm; elution with H_2O; 10 ml fractions). The compound eluted first was crystallized from EtOAc–EtOH–H_2O giving 5.4 mg polypodine B (**4**). Later fractions yielded after separation on alumina prep. TLC as above 9.5 mg ajugasterone C (**5**) and 5 mg of a new compound, 5-deoxykaladasterone (**7**). Fractions eluted with CH_2Cl_2–EtOH (85:15) were combined, the solvent was evaporated and the residue (5.6 g) was further purified on a polyamide column (150 g Polyamid Woelm; elution with H_2O), the fractions containing one ecdysteroid were evapd and the material was crystallized from EtOAc–MeOH giving 0.76 g of 20-hydroxyecdysone (**1**).

Herb. Plant material (1.8 kg) was extracted with 40 l 70% MeOH. The extract was treated with Me_2CO (see above) and the residue (147 g) was first purified on a polyamide column (400 g

Table 1. Chromatographic properties of the various ecdysteroids

Compound	TLC R_f* System 1	TLC R_f* System 2	HPLC R_t (min)† System 3	HPLC R_t (min)† System 4
1	0.34	0.18	53.2	7.3
2	0.44	0.34	22.0	12.0
3	0.49	0.46	17.2	16.9
4	0.32	0.27	29.3	7.5
5	0.35	0.21	33.3	15.0
6	0.39	0.47	20.4	11.3
7	0.56	0.67	10.4	20.0
Ecdysone			30.8	13.1
20-Hydroxy-ecdysone			53.2	7.3

*TLC on silica plates; System 1 = EtOAc–MeOH–NH_3 (85:15:5); System 2 = CH_2Cl_2–EtOH (85:15).

†System 3 = Silica column (Zorbax®-SIL, 250 mm long, 9.2 mm i.d.), isocratic solvent CH_2Cl_2–*iso*-PrOH–H_2O (125:25:2); flow-rate 4 ml/min. System 4 = Reverse-phase column (Spherisorb®-ODS 250 mm long, 4.6 mm i.d.), solvent: linear gradient from 20% to 50% CH_3CN in 0.1% trifluoroacetic acid (TFA) in water in 40 min, flow-rate 1 ml/min.

Polyamid Woelm; elution with H_2O then with MeOH). The H_2O fractions containing the ecdysteroids (108 g) were fractionated on an Al_2O_3 column with a CH_2Cl_2–EtOH gradient. 20-Hydroxyecdysone (1.1 g), 24(28)-dehydromakisterone A (3 mg), makisterone C (8 mg) and carthamosterone (1.3 mg) were obtained in a similar manner as for the roots.

Identification of the isolated ecdysteroids. 20-Hydroxyecdysone (**1**). The identity of the isolated compound with 20-hydroxyecdysone is based on HPLC, MS and NMR data (see Tables 1 and 2).

24(28)-Dehydromakisterone A (**2**) [12]. Isolated as white needles from EtOAc–MeOH, mp. 245–247°. UV λ_{max}^{EtOH} nm: 242 MS gave a M_r of 492 (makisterone A = 494). The presence of ions at m/z 363 and 345 was indicative of a steroid nucleus identical with that of 20-hydroxyecdysone. ^{1}H NMR spectrometry (1D and 2D COSY experiments) showed no difference with 20-hydroxyecdysone concerning the steroid nucleus. Two new signals appeared in the ethylenic area (δ4.95 and 5.13) which were coupled to two new signals (δ2.18 and 2.42) with a small coupling constant, as

Table 2. The ^{1}H NMR spectra of compounds **1**–**7** in CD_3OD

	1	**2**	**3**	**4**	**5**	**6**	**7**
1-Ha	1.43	1.43	1.43	1.68	1.39	1.43	1.7
1-He	1.78	1.79	1.79	1.78	2.59 (*dd*, 13, 4.2)	1.78	2.1
2-Ha	3.83 (m, $W_{1/2}=22$)	3.84*	3.83*	3.94	4.01	3.82	3.72
3-He	3.94 (m, $W_{1/2}=8$)	3.95*	3.94*	3.99	3.96	3.94	3.84
4-Ha	1.65	1.65	1.65	1.75	1.72	1.65	1.6
4-He	1.75	1.75	1.75	2.08	1.89	1.75	1.78
5-H	2.38 (*dd*, 12, 5)	2.38	2.37	—	2.35	2.4	2.40
7-H	5.85 (*d*, 2.5)	5.81*	5.80*	5.85*	5.80*	5.81*	5.75 (*br s* $W_{1/2}=4$)
9-Ha	3.14 (m, $W_{1/2}=22$)	3.16*	3.16*	3.19*	3.15 (*dd*, 8.8, 2.8)	3.15*	—
11-Ha	1.65	1.66	1.66	1.7	4.07 (m, $W_{1/2}=28$)	1.67	6.3 (*ddd*, 6.5, 2, 2)
11-He	1.78	1.79	1.79	1.8	—	1.80	—
12-Ha	2.13 (*ddd*, 13, 13, 5)	2.13	2.13	2.13	2.22	2.13	2.72 (*br d* 18)
12-He	1.85	1.87	1.87	1.88	2.16	1.85	2.42
15-Ha	2.00	1.98	2.00	2.00	1.95		
15-Hb	1.55	1.62	1.55	1.60	1.58		
16-Ha	1.95	2.0	2.0	2.0	1.98	2.05	2.05
16-Hb	1.75	1.82	1.77	1.75	1.74	1.75	1.75
17-H	2.39 (m)	2.39	2.39	2.39*	2.41*	2.38	2.45
22-Hb	3.33 (*dd*, 11, 2)	3.60 (*dd*, 10.4, 1.8)	3.42	3.33*	3.32*	3.70 (*dd*, 10.5, 2)	3.33*
23-Ha	1.30	2.18 (m)	1.43	1.28	1.22	2.57 (*ddd*, 17.5, 2, 2)	1.35
23-Hb	1.65	2.42 (m)	1.55	1.66		2.28	1.55
24-Ha	1.75	—	1.45	1.75-1.8		—	
24-Hb	1.45	—	—	1.45		—	
25-H	—	—	—	—	1.55	—	1.55
18-Me	0.89(*s*)	0.90*	0.89*	0.89*	0.88*	0.91*	0.89*
19-Me	0.96 (*s*)	0.97*	0.96*	0.92*	1.06*	0.97*	1.1*
21-Me	1.18 (*s*)	1.24*	1.20*	1.20*	1.20*	1.27*	1.18*
26-Me	1.19 (*s*)	1.32	1.10*	1.19*	0.92 (*d*, 6.4)	1.48*	0.91 (*d*, 6.5)
27-Me	1.20 (*s*)	1.38	1.21	1.19*	0.93 (*d*, 6.4)		0.94 (*d*, 6.5)
28	—	$=CH_2$ 5.13 ($W_{1/2}=2$) 4.95 ($W_{1/2}$ 2.8)	$-CH_2-$ 1.14 1.53	—		$=CH-$ 5.92 (*s*)	—
29	—	—	$-Me$ 1.01 (*t*, 6.5)	—	—	—	—

*Same value as in column **1**.

observed in 2D COSY. The latter signals were coupled together by a gem constant and were also coupled to the downfield-shifted 22-H signal: they were therefore assigned to 23-H signals. The 26- and 27-methyl signals were shifted downfield. All the preceding points were in agreement with the presence of a 24-methylene substituent in the C-24 position.

Makisterone C (**3**) [29, 30]. Isolated as white needles from EtOAc–MeOH, mp 266–270°. UV λ_{max}^{EtOH} nm: 242. MS gave a M_r of 508 (480 + 28); the spectrum gave the same fragments at *m/z* 363 and 345 as for 20-hydroxyecdysone, resulting from C20-C22 cleavage; EI-MS gave prominent ions at *m/z* 189, 171, 145, 127, which are consistent with a side-chain substituted at C-24 with an ethyl group [31]. ^{1}H NMR spectrometry showed no difference with respect to 20-hydroxyecdysone in the chemical shifts of protons of the ABCD rings. The 22-H signal was coupled to two 23-H and the 26- and 27-methyl groups appeared as singlets, as is the case when ecdysteroids bear a 25-OH; the 26-methyl was shifted upfield (δ 1.10). A new triplet signal at δ 1.01, was assigned to the methyl group of a 24-ethyl substituent in the C-24 position. This substitution is in agreement with the prévious points and was confirmed by 2D-COSY experiment.

Polypodine B (**4**) [32]. The isolated compound (white microcrystals, mp 253–256°) comigrated both on NP and RP-HPLC with an authentic sample (a gift of Dr D. Guédin, Strasbourg). UV λ_{max}^{EtOH} nm: 241. Its MS was identical with that of an authentic reference sample and gave a M_r of 496, with characteristic ions at *m/z* 379 and 361 consistent with a 20,22 diol and an additional -OH group on the steroid nucleus with respect to 20-hydroxyecdysone. From 1D and 2D COSY ^{1}H NMR spectroscopy it was obvious that the major differences with 20-hydroxyecdysone concerned only the signals from ring A. The disappearance of the 5-H signal, the large downfield shifts of the 1-H_{ax} (b) and 4-H_{eq} (b) and small effects on 2-H_{ax}, 3-H_{eq}, 4-H_{ax}, 7-H, 9-H and 19-Me were in agreement with the presence of a 5β-OH.

Ajugasterone C (**5**) [33]. Identified by comparison with an authentic sample from Dr Kubo (Berkeley, U.S.A.) using HPLC, MS and ^{1}H NMR (Table 2).

REFERENCES

1. Bergamasco, D. R. and Horn, D. H. S. (1983) in *Invertebrate Endocrinology* Vol. **1**. (Downer, R. G. H. and Laufer, H. eds) p. 627. Alan R. Liss, New York.
2. Horn, D. H. S. and Bergamasco, D. R. (1985) in *Comprehensive Insect Physiology, Biochemistry and Pharmacology* Vol. **7**. (Kerkut, G. A. and Gilbert, L. I., eds) p. 185. Pergamon Press, Oxford.
3. Syrov, V. N. and Kurmukov, A. G. (1975) *Voprosy Farmakol. i Toxikol.*, p. 92. Tashkent.
4. Krasnov, E. A., Saratikov, A. S. and Yakunina, G. D. (1977) *Izv. Sib. Otd. Akad. Nauk SSSR Ser. Biol. Nauk* **1**, 93.
5. Dolphin, D. and Wick, A. (1977) *Tabulation of Infrared Spectral Data* p. 356. J. Wiley, New York.
6. Günther, H. (1980) *NMR Spectroscopy*, pp. 75, 113. J. Wiley, New York.
7. Canonica, L., Danieli, B., Ferrari, G., Haimova, M. A. and Krepinsky, J. (1973) *Experientia* **29**, 1062.
8. Canonica, L., Danieli, B., Ferrari, G., Krepinsky, J. and Haimova, M. (1977) *Gazz. Chim. Ital.* **107**, 123.
9. Mamatkhanov, A. U., Shamsutdinov, M. I. and Shakirov, T. T. (1979) *Khim. Prir. Soedin.* **5**, 667.
10. Yatsuk, Ja. K. and Segel, G. M. (1970) *Khim. Prir. Soedin.* **6**, 281.
11. Kholodova, Yu. D., Baltaev, U., Volovenko, V. O., Gorovits, M. B. and Abubakirov, N. K. (1979) *Khim. Prir. Soedin.* **2**, 171.
12. Baltaev, U., Gorovits, M. B., Rashkes, Ya, V. and Abubakirov, N. K. (1978) *Khim. Prir. Soedin.* **4**, 463.
13. Baltaev, U., Gorovits, M. B., Abdullaev, N. D., Yagudaev, M. R. and Abubakirov, N. K. (1977) *Khim. Prir. Soedin.* **6**, 813.
14. Baltaev, U., Gorovits, M. B., Abdullaev, N. D., Rashkes, Ya, V. and Yagudaev, M. R. (1978) *Khim. Prir. Soedin.* **4**, 457.
15. Novosel'skaya, I. L., Gorovits, M. B. and Abubakirov, N. K. (1975) *Khim. Prir. Soedin.* **11**, 429.
16. Zlatsny, I. L., Gorovits, M. B. and Abubakirov, N. K. (1979) *Khim. Prir. Soedin.* **2**, 175.
17. Arsenault, G. P., Biemann, K., Barksdale, A. W. and McMorris, T. C. (1968) *J. Am. Chem. Soc.* **90**, 5635.
18. Fujimoto, Y., Miyasaka, S., Ikeda, T., Ikekawa, N., Ohnishi E., Mizuno, T. and Watanabe, K. (1985) *J. Chem. Soc. Chem. Commun.* 10.
19. Kubo, I., Matsumoto, A. and Hanke, F. J. (1985) *Agric. Biol. Chem.* **49**, 243.
20. Guerriero, A., Traldi, P. and Pietra, F. (1986) *J. Chem. Soc. Chem. Commun.* 40.
21. Kubo, I., Matsumoto, A., Matsumoro, T. and Klocke, J. A. (1986) *Tetrahedron* **42**, 489.
22. Girault, J.-P. and R. Lafont, R. (1987) *J. Insect Physiol.* (in press).
23. Lafont, R., Sommé-Martin, G., Mauchamp, B., Maume, B. F. and Delbecque, J.-P. (1980) in *Developments in Endocrinology* Vol. **7** (Hoffmann, J. A. ed.), p. 45. Elsevier/North Holland, Amsterdam.
24. Lafont, R., Sommé-Martin, G. and Chambet, J.-C. (1979) *J. Chromatogr.* **170**, 185.
25. Lafont, R., Beydon, P., Mauchamp, B., Sommé-Martin, G., Andrianjafintrimo, M. and Krien, P. (1981) in *Regulation of Insect Development and Behaviour* (Sehnal, F., Zabza, A. and Cymborowski, B. eds), p. 125. Technical University of Wroclaw Press, Wroclaw.
26. Bax, A. and Freeman, A. (1981) *J. Magn. Reson.* **44**, 542.
27. Eich, G., Bodenhausen, G. and Ernst, R. R. (1982) *J. Am. Chem. Soc.* **104**, 3731.
28. Wagner, G. (1983) *J. Magn. Reson.* **55**, 151.
29. Galbraith, M. N., Horn, D. H. S., Porter, Q. N. and Hackney, R. J. (1968) *J. Chem. Soc. Chem. Commun* 971.
30. Imai, S., Fujioka, S., Murata, E., Sasakawa, Y. and Nakanishi, K. (1968) *Tetrahedron Letters* **36**, 3887.
31. Nakanishi, K. (1971) *Pure Appl. Chem.* **25**, 167.
32. Jizba, J., Herout, V. and Sorm, F. (1967) *Tetrahedron Letters* **51**, 5139.
33. Imai, S., Murata, E., Fujioka, S., Koreeda, M. and Nakanishi, K. (1969) *J. Chem. Soc. Chem. Commun.* 546.