47. Ammodoremin, an Epimeric Mixture of Prenylated Chromandiones from Ammoniacum

by **Giovanni Appendino*** and **Gian Mario Nano**

Dipartimento di Scienza e Tecnologia del Farmaco, Corso Raffaello 31, I-10125 Torino

and **Davide Viterbo** and **Giovanni De Munno**

Dipartimento di Chimica, Università della Calabria, I-87030 Arcavacata di Rende (CS)

and **Marco Cisero**

San Giorgio Flavors S.p.A., Via Fossata 114, I-10147 Torino

and **Giovanni Palmisano***

Dipartimento di Chimica Organica e Industriale, Via Venezian 21, I-20133 Milano

and **Manuela Aragno**

Dipartimento di Medicina e Oncologia Sperimentale, Corso Raffaello 30, I-10125 Torino

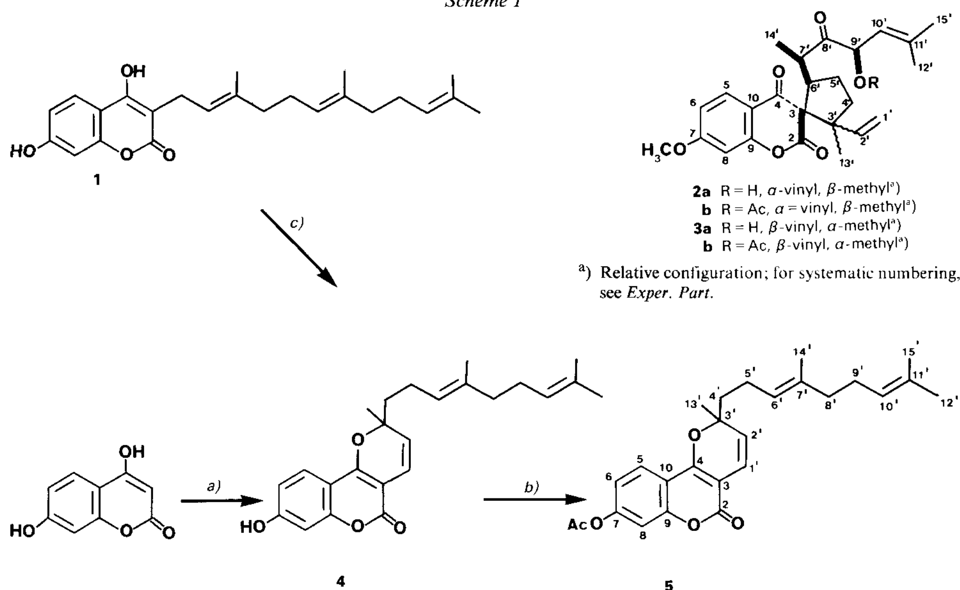
(17.XII.90)

From the gun resin Ammoniacum, an epimeric mixture **2a**/**3a** of highly functionalized prenylated chromandiones was isolated. Their structure was established by spectral data and single-crystal X-ray analysis. The compounds were isolated as acetates **2b**/**3b**, and these derivatives showed *in vivo* haemorrhagic activity.

Introduction. – The discovery of a series of prenylated 4-hydroxycoumarins and pyranocoumarins as the causative agents of ferulosys [1] prompted us to investigate a series of medicinal or alimentary gum resins known to contain prenylated coumarins and collectively referred to a 'fetid gums' or 'Kêma' [2]. Ammoniacum is one of these materials. The drug is obtained from *Dorema ammoniacum* DON. and some related plants from Central Asia and Persia [2], and its medicinal use was already known to the ancients, being documented in the Greek and Latin medical literature [2]. Early studies by *Späth et al.* [3] and *Kunz* and *Hoops* [4] culminated in the structure elucidation of the major phenolic constituent ammoresinol (**1**), but the interest in Ammoniacum was then scanty [5] [6], even though a recent patent claims antitumor properties for the crude gum resin [7]. We report here the isolation of a mixture of epimeric non-phenolic compounds occurring in Ammoniacum in a concentration comparable to that of **1**.

Results and Discussion. – Column chromatography of the powdered gum resin (commercial origin; see *Exper. Part*) gave in 11.7% yield a 5:4:1 mixture (NMR analysis) of the epimeric chromandiones **2a** and **3a**, which we call ammodoremin, and of the pyranocoumarin 7-hydroxyferprenin (**4**). This mixture was homogeneous by TLC and HPLC in several solvent systems. Attempts to remove the phenolic pyranocoumarin **4** by extraction with alkalies led to considerable decomposition of the chromandiones, but pyranocoumarin **4** could be easily separated after acetylation (Ac_2O, pyridine, room

Scheme 1



a) *trans,trans*-Farnesal, ethylenediammonium diacetate, MeOH (79%). *b*) Ac_2O, pyridine (82%). c) DDQ, THF (50%).

temperature). The acetate **5** obtained in this way from **4** was racemic, and its structure was confirmed by synthesis from *trans,trans*-farnesal (= (*E,E*)-3,7,11-trimethyldodeca-2,6,10-trienal) and 4,7-dihydroxycoumarin (= 4,7-dihydroxy-2*H*-[1]benzopyran-2-one), as outlined in *Scheme 1*. The synthesis relies on a chemoselective tandem *Knoevenagel* electrocyclic reaction already applied to the synthesis of other pyranocoumarins [8]. Compound **4** could also be obtained from the 4,5-dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile (DDQ) oxidation of ammoresinol (**1**).

The mixture **2b**/**3b** of the epimeric acetates was an optically active crystalline material. Its spectral analysis showed the presence of a methoxylated chromandione moiety and some structural units (vinyl and α-hydroxydimethylallyl groups, secondary and tertiary Me groups, keto group) that could be assembled in several ways, although only the formula corresponding to structures **2b** and **3b** seemed biogenetically plausible. Especially diagnostic features were the ^{13}C-NMR resonances of the spirane C-atom (*ca.* 72 ppm), an unusual chemical shift value for a sp^3-C-atom not bearing heteroatoms, and those of the two keto groups (*ca.* 209 (aliphatic ketone), 189 (aromatic ketone) ppm). The major differences in the ^{13}C-NMR spectra of **2b** and **3b** were observed for the frequences of the tertiary Me and the vinyl group, suggesting that the two compounds were probably epimers differing in the relative orientation of these two groups. Attempts to obtain the natural compounds **2a**/**3a** by hydrolysis of the acetate mixture **2b**/**3b** gave mixtures of products under a variety of experimental conditions.

Conclusive proof of the structure of ammodoremin (**2a**/**3a**) could be obtained only by X-ray analysis of the acetates **2b**/**3b**, that also established the relative configuration at the four non-epimeric centres (*Fig.*). The crystal is a solid solution of the two epimers at C(3′)

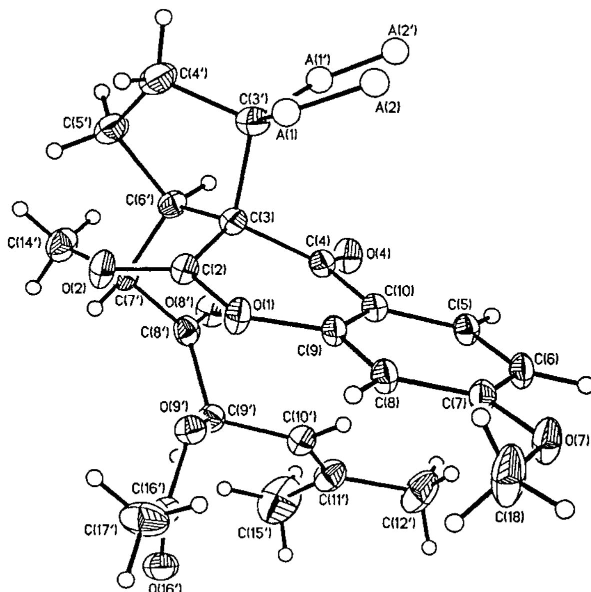


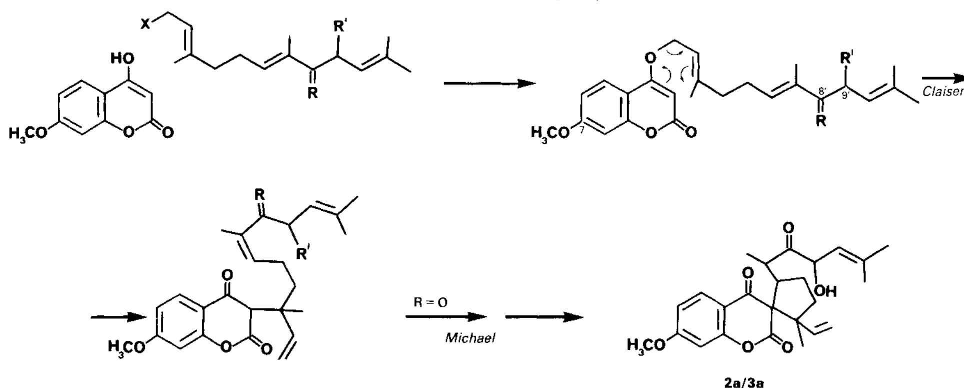
Figure. *ORTEP-II drawing of ammodoremin acetate* (**2b**/**3b**) *with thermal ellipsoids at the 20% probability level.* For systematic numbering, see *Exper. Part.*

(biogenetic numbering, *vide infra*), where all atoms, with the exception of the tertiary Me and the vinyl group, occupy the same position in the cell; A(1) and A(1′) in the *Figure* indicate the superposition of the Me C-atom and the vinyl CH group, while A(2) and A(2′) indicate almost half-populated vinyl CH_2 groups. The occupation factor of A(2) and A(2′) is set to 0.54 and 0.46, respectively, on the basis of ^{1}H-NMR analysis of the very same crystal used for the X-ray determination. Integration of the MeO signals shows in fact a ratio of 100:85. Values ranging from 100:80 to 90:100 are found in other crystals, thus implying a certain degree of variability in the crystal packing. The bond distances and angles do not show any abnormal values, except in the disordered region (atoms C(3), C(3′), A(1), A(2), A(1′), and A(2′)). The five-membered ring adopts an envelope conformation with C(3) as the flap and the pyrane ring a sofa conformation with C(3) out of the mean plane through the remaining atoms (*Table*). As to the relative configuration at C(3′), the set of ^{1}H-NMR signals corresponding to the major compound was assigned to **2b**, the epimer having a *cis*-relationship between the tertiary Me group and the C(6′)-side chain, on the basis of NOE experiments (NOE between H–C(6′) and Me(13′) in **3b**) and chemical-shift considerations (upfield resonance of the tertiary Me group). The absolute configuration could not be established.

Table. *Relevant Endocyclic Torsion Angles* [°] *of Ammodoremin Acetate* (**2b**/**3b**). E. s. d.'s in parenthesis.

C(6′)–C(3)–C(3′)–C(4′)	40.4(4)	C(10)–C(9)–O(1)–C(2)	7.0(5)
C(3)–C(3′)–C(4′)–C(5′)	– 27.4(5)	C(9)–O(1)–C(2)–C(3)	– 11.7(5)
C(3′)–C(4′)–C(5′)–C(6′)	3.9(5)	O(1)–C(2)–C(3)–C(4)	30.3(5)
C(4′)–C(5′)–C(6′)–C(3)	22.7(4)	C(2)–C(3)–(C4)–C(10)	– 31.8(4)
C(5′)–C(6′)–C(3)–C(3′)	– 38.9(4)	C(3)–C(4)–C(10)–C(9)	16.0(5)
C(4)–C(10)–C(9)–O(1)	4.3(5)		

Scheme 2. *Possible Biogenetic Route to Ammodoremin* (**2a**/**3a**). X = Leaving group; R = O- or 2 H-atoms; R′ = H-atom or OH group.



Probably **2a**/**3a** are formed from a dioxygenated coumarin and a farnesol derivative *via* O^4-alkylation, *Claisen* rearrangement, introduction of the oxygenated functions at C(8′) and C(9′), and *Michael* reaction (*Scheme 2*). The precise stage at which the methylation at O–C(7) and the oxygenation at C(8′) and C(9′) occur is not known.

Preliminary studies on the biological activity of **2b**/**3b** showed that these compounds display *in vivo* haemorrhagic activity at considerably higher doses than ferulenol, the major toxin from *Ferula communis* L. [1c], and warfarin. A significative increase of the prothrombin time could be observed only at doses 70 and 8 times, respectively, higher than those required to obtain a similar response with warfarin and ferulenol. Liver necrogenic effects were also evident only at the highest concentration tested (see *Exper. Part*).

Only very few examples of naturally occurring 2,4-chromandiones have been reported in the literature [9]. Furthermore, the spirojunction of a terpene unit to a non-isoprenoid moiety seems to represent a novel structural motif in the field of meroterpenoids of plant origine.

This work was supported by the *Ministero della Pubblica Istruzione* (fondi 40 and 60%).

Experimental Part

General. Column chromatography (CC): silica gel *60* (*Merck*, 70-230 mesh). TLC: precoated silica gel *60* F_{254} plates (*Merck*); detection with UV light (254 nm) or 5% H_2SO_4 in MeOH and heating. M.p.: *Büchi-SMP-20* apparatus (uncorrected). UV (λ_{max} in nm): *Beckman-DB-GT* spectrophotometer; in EtOH. IR (in cm^{-1}): *Perkin-Elmer-237* grating spectrometer. ^1H- and ^{13}C-NMR spectra: *Bruker-AC300* apparatus, 300 and 75.47 MHz, resp.; chemical shifts in ppm downfield from TMS (= 0 ppm): EI-MS: *Varian-MAT-CH-7A* spectrometer (70 eV).

Isolation of the Mixture of Ammodoremin (**2a**/**3a**) *and 7-Hydroxyferprenin* (**4**). Powdered Ammoniacum (240 g; Minardi, Bagnocavallo (RA), Italy) was charged on a silica-gel column (400 g) packed with hexane. Fractions of 250 ml were taken. The column was eluted with hexane containing increasing amounts of AcOEt. Hexane AcOEt 9:1 eluted 8.05 g of 2-nor-1,2-secoammoresinol [5] [6], and hexane AcOEt 8:2 gave 28 g (11.7%) of a 5:4:1 mixture of **2a**/**3a**/**4** as a yellowish gum. Further elution yielded 32 g of crude ammoresinol (**1**), that was purified using the method of *Späth et al.* [3].

Acetylation of **2a**/**3a**/**4**. A soln. of **2a**/**3a**/**4** (5:4:1; 24 g) in pyridine 150 ml was cooled to 0° (ice-bath). After addition of (150 ml) Ac_2O and stirring for 24 h at r.t., the mixture was poured into ice (*ca.* 500 ml), and MeOH (50

ml) was added. After 30 min, the mixture was extracted with CH_2Cl_2 (3×100 ml). The org. phase was washed with sat. $NaHCO_3$, soln. H_2O, dil. HCl, soln., and brine, dried ($MgSO_4$), and evaporated. The pasty residue was chromatographed (silica gel (100 g), hexane/AcOEt 6:4, 10-ml fractions). *Fr. 19–22* gave 1.3 g of **5** and *Fr. 33–88* crude **2b/3b** that was crystallized from boiling hexane. The precipitate was washed with hexane Et_2O: 8.17 g of **2b/3b**. Colourless powder. Large crystals suitable for X-ray analysis were obtained dissolving the powder in hot acetone and allowing the resulting soln. to cool in a *Dewar* flask containing H_2O at *ca.* 50°.

*7-Acetoxyferprenin (= 8-Acetoxy-2-(4,8-dimethylnona-3,7-dienyl)-2-methyl-2*H*,5*H*-pyrano[3,2-*c*][1]benzopyran-5-one*; **5**). Colourless oil, optically inactive. UV (EtOH): 345, 250, 227. IR (liq.): 1780, 1720, 1640, 1610, 1440, 1370, 1200, 1040, 1030, 905. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.80 (br. *d*, *J* = 8.6, H–C(5)); 7.08 (br. *s*, H–C(8)); 7.05 (br. *d*, *J* = 8.6, H–C(6)); 6.56 (*d*, *J* = 10.2, H–C(1')); 5.48 (*d*, *J* = 10.2, H–C(2')); 5.08 (*m*, H–C(6'), H–C(10')); 2.34 (*s*, AcO); 1.67 (*s*, Me(12')); 1.58, 1.56 (2*s*, Me(14'), Me(15')); 1.51 (*s*, Me(13')). ^{13}C-NMR (75.47 MHz, $CDCl_3$): 160.65 (*s*, C(2)); 99.48 (*s*, C(3)); 158.65 (*s*, C(4)); 124.13 (*d*, C(5)); 117.96 (*d*, C(6)); 153.37, 153.72 (2*s*, C(7), C(9)); 110.14 (*d*, C(8)); 115.47 (*s*, C(10)); 123.03 (*d*, C(1')); 117.08 (*d*, C(2')); 83.32 (*s*, C(3')); 41.73 (*t*, C(4')); 22.36 (*t*, C(5')); 123.03, 125.07 (2*d*, C(6'), C(10')); 131.34, 135.98 (2*s*, C(7'), C(11')); 39.55 (*t*, C(8')); 26.55 (*t*, C(9')); 25.61 (*q*, C(12')); 27.45 (*q*, C(13')); 15.95, 17.57 (2*q*, C(14'), C(15')). EI-MS: no M^+ ($C_{26}H_{30}O_5^+$), 362 (5, $[M - 60]^+$), 353 (5), 271 (58), 229 (100). Anal. calc. for $C_{26}H_{30}O_5$: C 73.91, H 7.16; found: C 72.85, H 7.19.

*Ammodoremin Acetate (= (1'*R**,2'*S**,5'*S**)- and (1'*R**,2'*R**,5'*S**)-5'-[(1''*S*,3''*S*)-3''-Acetoxy-1'',5''-dimethyl-2''-oxohex-4''-en-1''-yl]-7-methoxy-2'-methyl-2'-vinylspiro[2*H*-[1]benzopyran-3(4*H*)-1'-cyclopentane]-2,4-dione*; **2b/3b**). Colourless crystals (acetone). M.p. 119–120°. $[\alpha]_D^{25} = +154$ (CH_2Cl_2, *c* = 1.3). UV (EtOH): 281, 235, 209. IR (KBr): 3090, 3060, 1765, 1750, 1670, 1610, 1580, 1440, 1305, 1280, 1245, 1220, 1160, 1070, 860, 835. ^{1}H-NMR (300 MHz, $CDCl_3$): **2b**: 7.82 (*d*, *J* = 8.7, H–C(5)); 6.73 (*dd*, *J* = 8.7, 2.5, H–C(6)); 6.55 (*d*, *J* = 2.5, H–C(8)); 5.80 (*d*, *J* = 9.8, H–C(9')); 5.65 (*dd*, *J* = 15.2, 10.3, H–C(2')); 5.05 (br. *d*, *J* = 9.8, H–C(10')); 4.90 (*m*, 2 H–C(1')); 3.87 (*s*, MeO); 3.42 (*m*, H–C(6')); 3.15 (*m*, H–C(7')); 2.30 (*m*, 1 H–C(4')); 2.20 (*m*, 1 H–C(5')); 1.97 (*s*, AcO); 1.85 (*m*, 1 H–C(5')); 1.79 (br. *s*, Me(15')); 1.77 (*s*, Me(12')); 1.50 (*m*, 1 H–C(4')); 1.24 (*d*, *J* = 7.3, Me(14')); 0.98 (*s*, Me(13')); **3b**: 7.78 (*d*, *J* = 8.7, H–C(5)); 6.75 (*dd*, *J* = 8.7, 2.5, H–C(6)); 6.61 (*d*, *J* = 2.5, H–C(8)); 5.83 (*d*, *J* = 9.8, H–C(10')); 4.90 (*m*, 2 H–C(1')); 3.88 (*s*, MeO); 3.32 (*m*, H–C(6')); 3.15 (*m*, H–C(7')); 2.30 (*m*, 1 H–C(4')); 2.20 (*m*, 1 H–C(5')); 1.98 (*s*, AcO); 1.79 (br. *s*, Me(15')); 1.77 (br. *s*. Me(12')); 1.23 (*s*, *J* = 7.3, Me(14')); 1.07 (*s*, Me(13')). ^{13}C-NMR (75 MHz, $CDCl_3$)[1]): **2b**: 168.26 (*s*, C(2)); 71.86 (*s*, C(3)); 188.95 (*s*, C(4)); 128.50 (*d*, C(5)); 111.71 (*d*, C(6)); 165.82 (*s*, C(7)); 100.74 (*d*, C(8)); 156.57 (*s*, C(9)); 114.16 (*s*, C(10)); 115.52 (*t*, C(1')); 140.89 (*d*, C(2')); 56.03 (*s*, C(3')); 35.91 (*t*, C(4')); 28.54 (*t*, C(5')); 45.05 (*d*, C(6')); 45.55 (*d*, C(7')); 208.88 (*s*, C(8')); 73.93 (*d*, C(9')); 116.45 (*d*, C(10')); 142.85 (*s*, C(11')); 18.77 (*q*, C(12')); 21.93 (*q*, C(13')); 15.77 (*q*, C(14')); 25.94 (*q*, C(15')); 55.78 (*q*, MeO); 169.85, 20.65 (*s*, *q*, AcO); **3b**: 168.37 (*s*, C(2)); 71.60 (*s*, C(3)); 189.17 (*s*, C(4)); 128.89 (*d*, C(5)); 111.84 (*d*, C(6)); 165.82 (*s*, C(7)); 100.74 (*d*, C(8)); 156.54 (*s*, C(9)); 114.16 (*s*, C(10)); 112.80 (*t*, C(1')); 141.59 (*d*, (C(2')); 56.30 (*s*, C(3')); 36.41 (*t*, C(4')); 29.01 (*t*, C(5')); 45.47 (*d*, C(6')); 46.06 (*d*, C(7')); 209.09 (*s*, C(8')); 73.93 (*d*, C(9')); 116.49 (*d*, C(10')); 142.85 (*s*, C(11')); 18.77 (*q*, C(12')); 23.10 (*q*, C(13')); 15.60 (*q*, C(14')); 25.94 (*q*, C(15')); 55.78 (*q*, MeO); 169.85, 20.65 (*s*, *q*, AcO). EI-MS: 468 (0.8, M^+, $C_{27}H_{32}O_7^+$), 410 (10), 341 (75), 259 (100), 205 (45), 145 (40), 85 (85). Anal. calc. for $C_{27}H_{32}O_7$: C 69.23, H 6.83; found: C 69.17, H 6.74.

Synthesis of **5**. 1. *From Farnesal and 4,7-Dihydroxycoumarin.* To a soln. of 4,7-dihydroxycoumarin [10] (1.04 g, 5.8 mmol, 1.2 mol-equiv.) and ethylenediammonium diacetate (52 mg, 0.29 mmol, 0.05 mol-equiv.) in MeOH (20 ml), a soln. of *trans,trans*-farnesal (1.07 g, 4.87 mmol; prepared by MnO_2 oxidation of *trans,trans*-farnesol (*Aldrich*)) in MeOH (10 ml) was added dropwise during 5 min. After stirring 3 h at r. t., the solvent was evaporated. The residue was mixed with silica gel *40* (35–70 mesh, *Merck*) and separated on a short column of silica gel (70–230 mesh, 20 g, packed in hexane; elution with hexane, then hexane/AcOEt 6:4): 1.45 g (79%) of 7-hydroxyferprenin (**4**) as a yellowish oil. Part of this material (477 mg, 1.25 mmol) was treated with pyridine Ac_2O 1:1 (8 ml). After stirring overnight at r.t., the mixture was poured into ice and extracted with CH_2Cl_2. After washing with sat. $NaHCO_3$ and dil. HCl soln., the org. phase was dried ($MgSO_4$) and evaporated. The reddish residue was purified by CC (silica gel (4 g), hexane/AcOEt 9:1): 437 mg (82%) of **5**. Yellowish oil. Identical (^{1}H-NMR, IR) to the compound obtained from natural **4**.

2. *From Ammoresino* (**1**). To a stirred soln. of **1** (824 mg, 2.1 mmol) in THF (15 ml), DDQ (524 mg, 2.3 mmol, 1.1 mol-equiv.) was added. The soln. was stirred under N_2 for 5 min, evaporated, and purified by CC (silica gel (15 g), hexane/AcOEt 8:2): 414 mg (50%) of **4**. Acetylation as described above gave a compound identical (^{1}H-NMR, IR) to that obtained from natural **4**.

[1]) Multiplicities from the DEPT spectrum; assignments from chemical-shift and multiplicity considerations and from the ^{13}C,^{1}H-COSY spectrum.

X-Ray Analysis. Diffraction data were collected at r.t. on a *Siemens-R3m/V* four-circle diffractometer equipped with graphite monochromator. The intensities of a total of 2504 reflexions (2308 independent) were measured by $\theta/2\theta$ scan with variable speed. The cell parameters were obtained and refined from the angular setting of 23 reflexions in the range $15 \leqslant 2\theta \leqslant 30°$. The intensities were corrected for background and *Lorentz* polarization, but not for absorption effects. All calculations were carried out by the SHELXTL PLUS system [11]. The structure was solved by direct methods and refined by full-matrix least-squares techniques with anisotropic temperature factors for all non-H-atoms except those affected by disorder. H-Atoms were located in geometrically calculated positions and refined using a riding model with fixed isotropic temperature factors.

Crystal Data: $C_{27}H_{32}O_7$, M = 468.5, monoclinic, space group $P2_1$, $a = 8.927(3)$, $b = 13.534(4)$, $c = 10.602(3)$ Å, $\beta = 104.72(2)°$, $V = 1238.9(7)$ Å^3, Z = 2, $D_c = 1.256$ Mg/m^3, Mo$K\alpha$ radiation ($\lambda = 0.71073$ Å), $\mu = 0.084$ mm^{-1}, $R = 0.047$, $R_w = 0.053$, Goof = 1.96 for 2099 observed intensities ($3 \leqslant 2\theta \leqslant 50°$) having $I > 2.5° \sigma(I)$, weighting function $w = (\sigma^2 (F) + 0.0008\ F^2)^{-1}$, maximum residual electron density $\Delta\varrho = 0.3$ eÅ^3 (close to atoms A(1′) and A(2′), indicating a possible conformational disorder, besides the configurational one).

Determination of Prothrombin Time and Hepatic Necrosis. Male rats of *Wistar* strain, weighing 180–200 g (*Nossan*, Correnzana, Milano, Italy) were kept on a chow diet [12] without added synthetic antioxidants (*Piccioni*, Milano, Italy). The animals were starved 12 h prior to any treatment and refed between the 7th and the 12th h following the intoxication. H_2O was available *ad libitum*. The animals were housed and treated in accordance to the standards laid down in the Council of Europe directives [13]. The mixture **2b/3b** was administrated intragastrically, dissolved in the minimal amount of MeOH and then mixed with mineral oil. Different concentrations were used (2.13, 106.8, 213.7, and 1068.4 mmol/kg). Control rats received the vehicle only. The animals (5 for each group) were treated between 8.00 and 9.00 a.m. for two consecutively days, and 24 h later, they were sacrified. Blood samples (1 ml) were collected by cardiac puncture, under Et_2O anaesthesia. Subsequently, the rats were sacrified by withdrawing blood from abdominal aorta. The prothrombin time was determined in the plasma, obtained from samples of cardiac blood, with the *Behring* kit (*Tromborel S, Behringwerk*, Marburg, Germany). Serum obtained from aortic blood was frozen at −20°, and the serum activity of sorbitol dehydrogenase (SDH), alanino-aminotransferase (ALT), and aspartate aminotransferase (AST) was assayed by the UV combination test supplied by *Boehringer* (Mannheim, Germany). Statistical analyses were carried out by the *Student's* 't' test. A statistically significative increase of the prothrombin time (> 100 s; control 12.4 ± 0.7 s) and of the release of the marker enzymes into the circulation (*ca.* 20%) could be observed only at the highest dose tested.

Note Added in Proof. – We have been informed that a compound identical to one of the epimeric constituent of ammodoremin has been isolated from 'ammoniacum' by another group (*G. Nasini*, personal communication, *Gazz. Chim. Ital.*, in press).

REFERENCES

[1] a) M. Aragno, S. Tagliapietra, G. M. Nano, G. Ugazio, *Res. Commun. Chem. Pathol. Pharmacol.* **1988**, *59*, 399; b) S. Tagliapietra, M. Aragno, G. Ugazio, G. M. Nano, *ibid*, **1989**, *66*, 333; c) G. Appendino, S. Tagliapietra, P. Gariboldi, G. M. Nano, V. Picci, *Phytochemistry* **1988**, *27*, 3619.

[2] a) A. Tschirch, E. Stock, in 'Die Harze', Bornträger Verlag, Berlin, 1933, Vol. II, pp. 156–211; b) I. Guareschi, in 'Commentario della Farmacopea Italiana', Unione Tipografica Editrice, Torino, 1897, Vol. II, pp. 273–276.

[3] E. Späth, A. F. J. Simon, J. Lintner, *Ber. Dtsch. Chem. Ges.* **1936**, *69*, 1656.

[4] K. Kunz, L. Hoops, *Ber. Dtsch. Chem. Ges.* **1936**, *69*, 2174.

[5] G. Cardillo, M. Cricchio, L. Merlini, *Ric. Sci.* **1968**, *38*, 804.

[6] G. Appendino, S. Tagliapietra, G. Cravotto, G. M. Nano, *Gazz. Chim. Ital.* **1989**, *119*, 385.

[7] M. Chekroun, fr. demande FR 2,502,008, 1982 (*CA* : **1983**, *98*, 40602c).

[8] G. Appendino, G. Cravotto, S. Tagliapietra, G. M. Nano, G. Palmisano, *Helv. Chim. Acta* **1990**, *73*, 1865.

[9] a) F. Bohlmann, C. Zdero, H. Franke, *Ber. Dtsch. Chem. Ges.* **1973**, *106*, 382; b) J. De Pasqual Teresa, M. A. Villaseco, J. M. Hernández, J. R. Morán, J. C. Urones, M. Grande, *Planta Med.* **1986**, 458; c) I. M. Lockhart, in 'The Chemistry of Heterocyclic Compounds, Chromenes, Chromanones and Chromones', Ed. G. P. Ellis, Wiley, Chichester, 1977, Vol. XXI, pp. 429–453.

[10] V. R. Shah, J. L. Bose, R. C. Shah, *J. Org. Chem.* **1960**, *25*, 677.

[11] SHELXTL PLUS Version 3.4′, *Siemens Analytical X-Ray Instruments Inc.*, Madison, WY, 1989.

[12] L. Randoin, T. Causeret, *Bull. Soc. Hyg. Aliment.* **1947**, *35*, 14.

[13] Council of Europe (1982), 'European Convention for the Protection of Vertebrate Animals used for Experiments and Other Scientific Purpose, Addendum III', Strasbourg, 1982, 9 July.