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SESQUITERPENE LACTONES. CONSTITUENTS OF *ARTEMISIA NOVA* NELS. AND *A. TRIPARTITA* GRAY SSP. *RUPICOLA*[1]

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Abstract—*Artemisia nova* and *A. tripartita* ssp. *rupicola* have been found to contain several guaianolides of closely related structures. One of them, cumambrin B, has been reported as a constituent of *Ambrosia cumanensis* and has also been found in this laboratory in *Ambrosia acanthicarpa*. The gross structure assigned to cumambrin B has been confirmed and its stereochemistry completely defined.

INTRODUCTION

Artemisia nova Nels. and *A. tripartita* Grey ssp. *rupicola* are members of the subgenus (Section) Seriphidium and of the "tridentatae" complex of the subgenus. They are closely allied to *A. tridentata* and have been found to contain several guaianolides that are closely related in structure to matricarin and its congeners, constituents of *A. tridentata* ssp. *tridentata*.[2]

A. nova and *A. tripartita rupicola* are quite similar in constitution and little variation was observed among several collections of each species. The most abundant compound in both is cumambrin B,[3] accompanied by smaller amounts of cumambrin A (8-*O*-acetylcumambrin B) and a new guaianolide, 8-deoxycumambrin B.

The structures of cumambrin B (I), cumambrin A (II), and 8-deoxycumambrin (III) can be compared with those of matricarin (V), deacetylmatricarin (IV), and deacetoxymatricarin (VI). The similarities of the gross structures of these compounds are evident; yet none of I, II, or III has been found in *A. tridentata*, nor IV, V or VI in *A. nova* or *A. tripartita rupicola*. Thus, although the members of the *tridentata* species[4] and *A. nova* and *A. tripartita* possess many morphological characteristics in common, the latter two are clearly distinguished from the former, but show a remarkable similarity to each other.

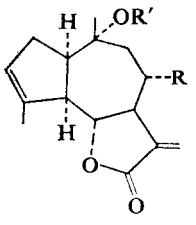
The structures of cumambrins A and B have been established in most details by Romo *et al.*[3] Our own studies have confirmed the structures proposed by these investigations and have completed the definition of the stereochemistry at all asymmetric centers.

[1] Contribution No. 2283 from the Department of Chemistry, U.C.L.A.

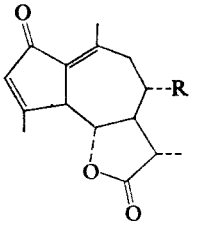
[2] T. A. GEISSMAN, T. STEWART and M. A. IRWIN, *Phytochem.* 6, 901 (1967).

[3] Cumambrins B and A, which while under study here were designated as artenovin and its acetate, respectively, were recently reported as constituents of *Ambrosia cumanensis* HBK. (J. ROMO, A. ROMO DE VIVAR and E. DIAZ, *Tetrahedron*, in press. We are grateful to Dr. Romo for a prepublication copy of his paper.) Although the physical properties of the cumambrins do not correspond exactly with those we observed for "artenovin" and its acetate, Dr. Romo has compared his compounds with specimens supplied by us and reports that they are identical.

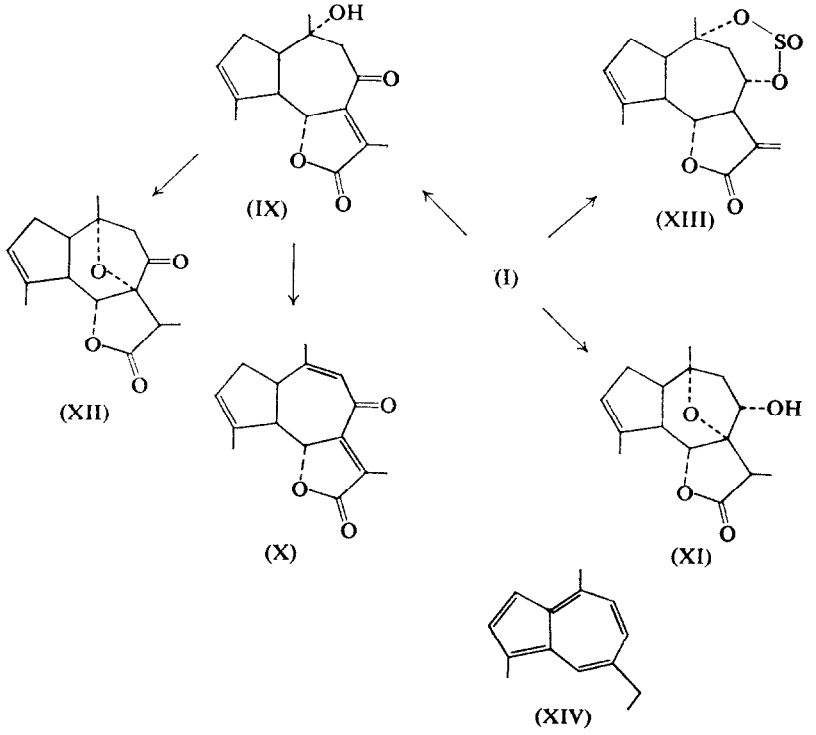
[4] *A. tridentata* ssp. *parishii* contains deacetoxymatricarin and differs from ssp. *tridentata* only in the relative amount of this compound.



(I) R = OH, R′ = H
(II) R = OAc, R′ = H
(III) R = H, R′ = H
(VII) R = OAc, R′ = Ac
(VIII) R = OCHO, R′ = CHO



(IV) R = OH
(V) R = OAc
(VI) R = H



RESULTS AND DISCUSSION

That cumambrin A was the monoacetate of cumambrin B was established by acetylation of the latter.[5] Cumambrin B (I), $C_{15}H_{20}O_4$, had i.r. and u.v. spectra in accord with main structural features shown in I. Its mass spectrum showed a molecular ion at *m/e* 264, with prominent ions at M-18 and M-36. Cumambrin A (II) showed, besides the molecular ion

[5] Cumambrin B is reported[3] to have m.p. 87°, $[\alpha]_D$ +81°. Our values for the compound (artenovin) were m.p. 178–180°, $[\alpha]_D$ +92·5°. Dr. J. Romo reported (private communication) that his cumambrin B appeared to crystallize in a solvated form, although we had not observed this. Cumambrin A was reported to have m.p. 178°, $[\alpha]_D$ +90°; our "artenovin acetate" had m.p. 188–190°, $[\alpha]_D$ +103°.

at *m/e* 308, a major ion at M-60; and the mass spectra of cumambrins A and B were substantially identical at *m/e* values below the M-18 (for B) and M-60 for (A) peaks.

The NMR spectrum of cumambrin B shows signals for the exocyclic methylene group at δ 6·03 and 6·19, coupled (3 Hz) to the proton at C-7. The C-4 methyl group is seen in the 3-proton signal at δ 1·91, coupled (1·5 Hz) to the vinylic proton at C-3, the signal of which is a broad singlet ($W_{1/2}$ 6 Hz) at δ 5·50.

The most significant feature of the NMR spectrum of I was the symmetrical triplet signal for the lactonic hydrogen at C-6. This appeared at δ 3·97, with two couplings of 9·5 Hz with the C-5 and C-7 protons. The signal for the proton at C-7 is obscured in the spectrum of I, but can be seen in the spectra of the diacetate (VII) and the diformate (VIII) of I, where it appears as a complex one-proton signal with coupling of 3 Hz with the protons of the exocyclic methylene group and 9·5 Hz with H-6. Although the signal for the H-8 proton is complex because of coupling with H-7 and the two protons at H-9, the couplings between the 5, 6, 7 and 8 hydrogen atoms are consistent with an all *trans*-axial arrangement. Thus, if the C-7 substituent be assigned the β-configuration possessed by this bond in all of the sesquiterpenes of the class, the configurations of the hydrogen atoms are 5α, 6β, 7α and 8β.

Oxidation of I yields the corresponding ketone (IX) in which the C-11/13 double bond has migrated to the 7/11 position, in conjugation with the carbonyl group. The product could be dehydrated to the trienone (X), the u.v. spectrum of which (λ_{max} 262 nm, ϵ 13,000) is in accord with the structure assigned.

Dehydrogenation of I gave chamazulene. This result, along with those described above, permitted the conclusion that I has the structure shown, in agreement with the proposal of Romo *et al.*[3] The stereochemistry at C-10 is not, however, defined by these observations, and was established by the reaction of I with thionyl chloride, with the formation of two cyclic thionyl esters (XIII), differing in the configuration of the asymmetric sulfoxide.

The configuration at C-1 was shown by the properties of two compounds formed by the isomerization of I and IX. The spectral characteristics of these compounds (XI from I; XII from IX) showed that they were formed by addition of the C-10 hydroxyl group to the double bond of the $\Delta^{7,11}$-lactonic function, and possess the structures shown. The NMR spectra of (XI) and (XII) differ from I and IX in that they possess a C-11 methyl group (3-proton doublet; 7 Hz). The H-6 signal at about δ 4·1 is a doublet with the small coupling constant of about 1 Hz. This coupling indicates a dihedral angle of about 110° between H-5 and H-6. A model of the compound with H-1 and H-5 *cis* (α) can accommodate a dihedral angle (H 5/6) of between 90° and 150° due to the flexibility of the system. The compound having H-1 β and H-5 α (ring junction *trans*) is both strained and inflexible, with a dihedral angle for H-5/6 of 180°. This would display a signal for H-6 with a coupling constant of about 9 Hz; in fact, the signal for H-6 in XI is nearly a singlet.

The formation of chamazulene from I proceeded with extraordinary ease.[6] When I was heated in glycerol solution containing sodium acetate, chamazulene was produced (62 mg from 267 mg of I). It was identified by conversion to the known trinitrobenzene adduct.

Numerous additional transformation products of I were prepared in the course of this work. Since the results described above, coupled with those of Romo *et al.* establish the structure and stereochemistry of the compound, these additional results are not described in detail.

[6] Romo *et al.* prepared an azulene from cumambrin B by lithium aluminum hydride reduction followed by palladium dehydrogenation at 295°.

8-Deoxycumambrin B (III) was isolated from both *A. nova* and *A. triparitita* ssp. *rupicola*. Although this compound was not obtained in sufficient quantity to permit an exhaustive study, its composition and spectral properties leave little doubt that it is indeed 8-deoxycumambrin. The compound has the composition $C_{15}H_{20}O_3$ and possesses a tertiary hydroxyl group. Its NMR spectrum shows the exocyclic methylene group (δ 5·45d, 6·12d; 3 Hz); the methyl groups at C-10 (δ 1·19S) and C-4 (δ 1·87d; 1·5 Hz); the vinyl proton at C-3 (δ 5·47, broad); and the lactonic proton at C-6 as a well-defined triplet (δ 4·12 tr; 9·5, 9·5 Hz). These signals correspond closely with those observed for I and its acetate, and the spectrum of III as a whole differs from that of I principally in the absence of the signals for the secondary hydroxyl function at C-8. Attempts to convert I and III into a common transformation product by removal of the oxygen function at C-8 of I have so far been unsuccessful because of the fact that crystalline compounds have not been obtained. The simple route that can be envisaged, of converting I into III by oxidation to the ketone and reduction of the dithioketal, cannot be performed for, it will be recalled, the oxidation of I to the ketone is accompanied by isomerization of the C-11/13 double bond to the 7/11 position. While this effort has not been abandoned, it is felt that the close correspondence in the relevant features of the NMR spectra of I and III, coupled with the co-occurrence of I, II and III, and of IV, V and VI in a closely allied species, strongly support the assignment of the indicated structure to 8-deoxycumambrin.

The mass spectra of I, II and III provide further support for the structure assigned to III. The mass spectra of I and II were discussed briefly above. In addition to the ions for M, M-18, M-36, M-60, etc., both mass spectra contain prominent ion peaks at the lower *m/e* values 121, 107, 105, 93, 91, 81, 80, 79, 77. The mass spectrum of 8-deoxycumambrin B shows, besides the peaks for the molecular ion (*m/e* 248), prominent peaks at M-18, M-18-15; and in the region of lower *m/e* values, peaks at *m/e* 121, 107, 105, 93, 91, 81, 80, 79 and 77. The correspondence between the ion peaks for I, II and III at the lower range of *m/e* values suggests that these ions represent identical fragments from the cyclopentane portion of the molecules, and that the differences between I, II and III are to be found in the nature of the cycloheptane moiety of this bicyclic system.

The occurrence of I in *Ambrosia cumanensis*[3] is paralleled by our discovery of the compound in a specimen of *Ambrosia acanthicarpa*.[7] The isolation of a guaianolide from *Ambrosia*, a genus that is characterized by the presence of numerous pseudoguaianolides, and the occurrence of the same guaianolide in *Ambrosia* and *Artemisia*, is of considerable taxonomic significance. While these genera are both composites, there are few morphological similarities between them. Payne[8] has suggested, however, that there is a close relationship between the tribes to which they belong (Ambrosieae and Anthemideae), a suggestion that is supported by these findings. No pseudoguaianolides have, however, yet been found in plants of the tribe Anthemideae.

EXPERIMENTAL

Melting points were determined in capillary tubes and are corrected. Spectra were determined as follows: NMR in $CDCl_3$ with TMS as internal standard; i.r.i n $CHCl_3$; u.v. in 95% ethanol. TLC was carried out on silica gel G, the usual solvent being chloroform–acetone (4:1). Spots were visualized by spraying with sulfuric acid and heating.

Relevant features of the NMR spectra are noted and described in the Discussion.

[7] T. A. GEISSMAN, SCOTT GRIFFIN, T. G. WADDELL and H. C. CHEN, *Phytochem.* **7**, 145 (1968). The occurrence of I in *A. acanthicarpa* was noted in this paper, but experimental details were omitted.

[8] W. W. PAYNE, *J. Arnold Arbor.* **45**, 401 (1964); and by private communication.

Extraction of Plant Materials

About ten separate collections of *Artemisia nova* and *A. tripartita rupicola* were examined. Two typical extractions are the following:

A. nova. A sample of dried leaves and flowers of *A. nova* (Voucher No. GHW-966-AN)[9] (370 g) was extracted with 4 × 2 l. of chloroform. The residue left after removal of the solvent was suspended in water and the mixture agitated with steam until the vapor temperature exceeded 90°. The mixture was allowed to cool and the aqueous phase carefully decanted from the tar. Five repetitions of this procedure, followed in each case by extraction of the aqueous solution with chloroform, afforded 8·8 g of crude chloroform-extractable material. This was chromatographed over 400 g of silica gel ("Baker Analyzed"), fractions of 500 ml being collected while eluting with chloroform (fractions 1–26) and chloroform containing increasing proportions of acetone (fractions 27–37).

Fractions 4 and 5 yielded crystalline material (0·48 g), crystallized from ethyl acetate as colorless granular crystals, m.p. 188–190°. This is cumambrin A (cumambrin B acetate) (II). Fractions 10–26 were evaporated to yield cumambrin B (I), colorless crystals from ethyl acetate, m.p. 178–180° (1·27 g).

A. tripartita ssp. *rupicola*

Two collections of *A. tripartita* ssp. *rupicola* (Vouchers ROA-8767A-ATR and ROA-8767B-ATR) totaling 3193 g were identical by TLC assay. They were combined and extracted with chloroform. The tarry residue obtained after removal of the solvent was extracted four times with a hot ethanol–water (3:1) mixture and the tar separated. Chloroform extraction of the clarified aqueous layer yielded 154 g of oily material. Chromatography of this crude product over silica gel, with chloroform and chloroform–acetone as eluting solvents, yielded 20 g of crystalline cumambrin B (I), and several other compounds which are still under investigation.[10] Cumambrin A (II) was present in these extracts (by TLC) but in a very small amount; it was not obtained as a crystalline product from this specimen.

Reworking of the tarry residues yielded a further 17 g of I, to give a total of 37 g (1·2 per cent).

The following is a summary of the results of other extractions (in per cent of dry plant material).

Cumambrin B (I)		*Cumambrin A (II)*	
AAB-81366-AN, AAB-81366-ATR	0·21	AAB-81366-AN	0·05
GHW-966-AN	0·34	GHW-966-AN	0·13
AAB-266-ATR	0·06	AAB-81366-ATR, GHW-966-ATR	0·01
GHW-966-ATR	0·44		

Cumambrin B (I) had m.p. 178–180°, $[\alpha]_D$ +92·5 (*c* 5·58, $CHCl_3$). I.r. peaks: 3579, 3503, 1762, 1659 cm^{-1}; u.v. max. 197 nm (ϵ 13,600). Mass spectrum, *m/e* 264 (M^+), 246, 228, 213 and lower *m/e* peaks described in the text. (Found: C, 68·10, 68·14, 68·26; H, 7·63, 7·75, 7·61. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63 per cent.)

Cumambrin A (II), from plant material or prepared by acetylation of I with acetic anhydride–pyridine, had m.p. 188–190°, $[\alpha]_D$ +103° (*c* 2·79, $CHCl_3$). I.r. peaks, 3575, 3450, 1763, 1659 cm^{-1}. Mass spectrum, *m/e* 306 (M^+), 288, 246, 228, 213 and lower *m/e* peaks described in the text. (Found: C, 66·76, 66·73, 66·74; H, 7·24, 7·17, 7·38. Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24 per cent.)

Cumambrin B Diacetate (VII)

To a solution of 500 mg of I in 100 ml of acetic anhydride was added a drop of conc. H_2SO_4. After a few minutes water was cautiously added and the solution diluted to 400 ml. Crystallization took place, and after it was complete the product was collected and recrystallized from ether–ethyl acetate to give 300 mg of colorless leaflets, m.p. 198–200°. I.r. peaks, 1765, 1733, 1656. Mass spectrum, *m/e* (no M^+ peak) 288 (M-60), 228 (M-60-60), 213. (Found: C, 65·81; H, 6·94. Calc. for $C_{19}H_{24}O_6$: C, 65·50; H, 6·94 per cent.)

The NMR spectrum showed two three-proton singlets (δ 2·03, 2·08) for the acetyl methyl groups. The lactonic proton at C-6 appeared as a triplet at δ 4·04 (J=9·5, 9·5 Hz), and the C-7 proton could be discerned as a multiplet, one coupling constant of which was 9·5 Hz.

Cumamarin B Diformate (VIII)

The diformate was isolated as a by-product of the formylation (of the C-8 OH group) of I with anhydrous formic acid. The 8-*O*-formyl compound had m.p. 175–180°; it is not described further. The diformate

[9] Voucher numbers refer to specimens placed in the U.C.L.A. Herbarium. We are indebted to Professor G. H. Ward, Knox College, Galesburg, Ill., and to Professors A. A. Beetle and R. O. Asplund, University of Wyoming, Laramie, for collections and authentication of the plant specimens studied.

[10] Most *Artemisia* species studied in the course of these investigations contain coumarins, some known and some not previously found as natural products. These will be described in separate communications.

(eluted earlier from a silica gel column) crystallized from ether–ethyl acetate as stout needles, m.p. 166–167·5°. (Found: C, 63·88; H, 6·24. Calc. for $C_{17}H_{20}O_6$: C, 63·74; H, 6·29 per cent.)

The diformate was of value chiefly because its NMR spectrum showed a well-defined signal for the C-7 proton, δ 3·55 (J=9·5, 3 Hz). The C-6 proton was a triplet with δ 4·07 (J=9·5, 9·5 Hz).

Dehydrocumambrin B (IX)

A solution of 500 mg of I in 20 ml of acetone was oxidized with a solution of chromic acid in acetic acid containing four equivalents of oxidant. After 12 hr at 0° the solution was diluted with water and extracted with chloroform. The chloroform solution was washed with aqueous sodium carbonate, dried, and evaporated. Chromatography of the oily residual material (silica gel, $CHCl_3$) yielded 200 mg of crystalline ketone, m.p. 133–134·5°. I.r. peaks, 3563, 3440, 1756, 1693, 1640. U.v. max. 244 nm (ε 10,900). Mass spectrum, *m/e* 262 (M^+), 244, 220, 219. (Found: C, 68·80; H, 6·85. Calc. for $C_{15}H_{18}O_4$: C, 68·68; H, 6·92 per cent.)

Anhydrodehydrocumambrin B (X)

A solution of 240 mg of IX and 50 mg of *p*-toluenesulfonic acid in 10 ml of isopropenyl acetate was allowed to stand for 14 hr and then washed with 10 ml of water. The solution was concentrated and filtered through a short column of silica gel and the product crystallized from ether–petroleum ether, giving 125 mg (plus a second crop of 89 mg) of the acetate of IX, m.p. 118·5–121°. I.r. peaks, 1756, 1699, 1640 cm^{-1}. Mass spectrum, *m/e* (no M^+), 244, 229, 226, 216, 215, 211.

When dehydrocumambrin B (IX) acetate was passed through a silica gel column, the elements of acetic acid were removed with the formation of anhydrodehydrocumambrin B (X), m.p. 177–179°. I.r. peaks, 1765, 1659, 1622 cm^{-1}. U.v. max. 262 nm (ε 13,000). Mass spectrum, *m/e* 244 (M^+), 229, 226, 216, 215, 211. (Found: C, 73·81; H, 6·68. Calc. for $C_{15}H_{16}O_3$: C, 73·75; H, 6·60 per cent.)

Isocumambrin B (XI)

A solution of 500 mg of I and 870 mg of sodium acetate in dimethylsulfoxide was heated briefly to boiling (2 min; longer heating produced chamazulene), cooled, diluted with sodium chloride solution and extracted with benzene. The extract (containing a trace of I, as shown by TLC) was evaporated to yield a residue that crystallized from ether–petroleum ether as fibrous needles, m.p. 192–202°. I.r. peaks, 3552, 3480, 1773 cm^{-1}. U.v., end absorption only. Mass spectrum, *m/e* 264 (M^+), 246, 228, 220, 213. (Found: C, 68·10; H, 7·70. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63 per cent.)

The NMR spectrum showed the absence of the exocyclic methylene group, and a signal for a secondary methyl group, δ 1·33d (J=6·5 Hz).

Isodehydrocumambrin B (XII)

A mixture of 123 mg of dehydrocumambrin B (IX), 50 mg of alumina (grade I, basic) and 5 ml of ethylene glycol was heated to boiling and immediately cooled, diluted with water and extracted with benzene. The extract was dried and evaporated and the residue chromatographed over silica gel. The product could not be crystallized, but gave a single spot on TLC. It was purified by distillation (short path) under vacuum (160°) and was obtained as a yellow oil. I.r. peaks, 1785, 1764 cm^{-1}. Mass spectrum, *m/e* 262 (M^+), 246, 224. (Found: C, 69·18; H, 6·95. Calc. for $C_{15}H_{18}O_4$: C, 68·68; H, 6·92 per cent.)

Cumambrin B Cyclic Sulfites-A and -B (XIII)

To a cold (0°) solution of 644 mg of I in 7 ml of pyridine was added 0·5 ml $SOCl_2$. After 5 min the solution was diluted with water, acidified with H_2SO_4, and extracted with a mixture of benzene and chloroform. The extract, which showed two spots on TLC, was chromatographed on silica gel with benzene–chloroform as eluant. The faster-moving component, sulfite-A (99 mg), was crystallized from ether, and formed granular crystals, m.p. 54–56°. I.r. peaks, 1769, 1664 cm^{-1}. Mass spectrum, *m/e* 310 (M^+), 246 (M-64), 228, 213. (Found: C, 58·88; H, 6·69. Calc. for $C_{15}H_{18}O_5S$: C, 58·06; H, 5·85 per cent.)

The sample could not be freed completely from a trace of solvent, for its low melting point precluded efficient drying; and it was quite unstable, decomposing with darkening and evolution of SO_2.

The slower-moving component of the mixture, sulfite-B, was obtained in later fractions from the column. It formed colorless crystals, m.p. 130–139° dec. (190 mg). I.r. peaks, 1771, 1664 cm^{-1}. Mass spectrum, *m/e* 310 (M^+), 246 (M-64), 228, 213. (Found: C, 58·12; H, 5·89. Calc. for $C_{15}H_{18}O_5S$: C, 58·06; H, 5·85 per cent.)

Both sulfite esters were unstable, deteriorating on keeping, with darkening and evolution of SO_2.

Chamazulene (XIV)

A mixture of 267 mg of I, 100 mg of NaOAc and 6 ml of glycerol was heated to boiling for 2·5 min. The solution turned green, then blue and a blue oil collected on the surface. The cooled mixture was diluted

with water and extracted with petroleum ether, and the extracted material distilled *in vacuo* to yield 62 mg of chamazulene as a deep-blue distillate. The trinitrobenzene complex formed fibrous, purple needles, m.p. 132–133° (reported,[11,12] m.p. 132°). (Found: C, 60·29; H, 4·97. Calc. for $C_{20}H_{19}O_6N_3$: C, 60·45; H, 4·82 per cent.)

8-Deoxycumambrin B (III)

A specimen of *A. nova* (Voucher ROA-73067-ANLW) (5000 g) was extracted and the extract processed as described above for the case of *A. tripartita rupicola.* Besides cumambrin B (I) (44 g) and its acetate (II) (15 g), chromatography, monitored by TLC examination, afforded 0·2 g of a new lactone, 8-deoxycumambrin B (III). Recrystallized from ether–petroleum ether it formed colorless prisms, m.p. 117–119°. I.r. peaks, 3572, 3431, 1761, 1668 cm^{-1}. The mass spectrum showed peaks at *m/e* 248 (M^+), 230 (M-18) and 215 (M-18-15), along with ion peaks at lower *m/e* values which have been described and interpreted in the text. The NMR spectrum has also been discussed earlier. (Found: C, 72·66; H, 8·15. Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12 per cent.)

Besides I–III, this specimen of *A. nova* afforded a sterol which was identified as β-sitosterol containing a small contaminating amount of stigmasterol (by NMR and mass spectra). Along with the constituents described above, this and other specimens of *A. nova* and *A. tripartita rupicola* contained a number of other sesquiterpenes and coumarins, of which several have been obtained in pure condition. Studies on these compounds are not yet complete.

Acknowledgements—This study was carried out with the aid of a research grant, GM-14240-02, from the U.S. Public Health Service. We thank Dr. J. Romo for providing us with information concerning the cumambrins. Elemental analyses are by Miss Heather King.

[11] K. Takeda, F. Sorm and V. Herout, *J. Pharm. Soc. Japan* **74**, 700 (1954).
[12] A. Meisels and A. Weizmann, *J. Am. Chem. Soc.* **75**, 3865 (1953).