Contribution to the study of *Eriocephalus punctulactus* essential oil: II. Azulenic compounds

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Essence of *Eriocephalus punctulactus* is obtained by steam distillation of the blue flowers of a bush which grows at 3000 meters on the slopes of the Lesotho mountains in South Africa. Two other species of *Eriocephalus—umbellatus* and *africanus*—are known for their essences from flowers and leaves.

E. punctulactus essence is characterized by a strong blue color and a powerful fruity, fresh odor, which reminds one of Roman chamomile. Its CPG spectrum, obtained on a Carbowax capillar column, shows more than 200 peaks, the most important not exceeding 15 percent. A primary study demonstrated that 55 percent of the essence could readily be separated by distillation of a colorless fraction.[1] This fraction contains the classical terpenes and esters, the most important of them being 2-methyl-isobutyl isobutyrate. Further study sought to identify in the remaining blue fraction the azulenic components which are generally responsible for the blue coloration of other essences (chamomile, millefolide). TLC revealed a blue component, the R_f value of which corresponds to that of an authentic sample of chamazulene.

The azulenic components of this oil are present in very low concentration, and they are easily degraded by light and air. For these reasons, a number of ways to isolate them were considered:

1. chemical separation: formation of additive compounds with strong acids[2]
2. separation by ion exchange resins: formation of acid resin of the tropylium cation, which is eluted with a water-alcohol solution to regenerate the azulene[3]
3. distillation: followed by GLC, liquid chromatography column

Experimentation with these different methods showed that best results could be obtained by distillation with a spinning band column, followed by separation by liquid chromatography and TLC to give a final purity greater than 95 percent.

By IR and PMR analysis 1,4-dimethylazulene and 1,4-dimethyl-7-ethylazulene were identified. A further UV study of the pure compounds determined the total azulene in the essence.

Separation

Distillation. A first distillation under reduced pressure yielded a colorless fraction (bp 40°-101° at 300 to 100 mm of Hg), which represented 55 percent by weight of the essence (550 g).[1] The remainder (447 g) was then distilled on a Podbielniac spinning band column. The distillation, lasting 192 hours, permitted the separation of 32 fractions (reflux ratio 1/90; pressure: 2-0.5 mm). The fractions 11, 12, and 13 (44 g; bp. 84°-88° at 1.7 mm) contained the first azulene (A), 8 percent. The fractions 29, 30, and 31 (33 g; bp 103°-106° at 1.7 mm) contained the second azulene (B), 2 percent.

Preparative liquid chromatography column. As the chromatographic characteristics of the two azulenes were identical, the study was continued using TLC and a chromatography column packed with silicagel or alumina. The conditions for the separation of the azulenes from their fractions were as follows:

Packed column: 70 cm x 25 mm ID
Silicagel: 0.04-0.063 mm
Solvent volume: 130 ml
Sample size: 10-11 g
Ascending elution with hexane
Elution time: 10 h

It must be noted that the azulenic compounds decomposed rapidly on silicagel, and this decomposition was accelerated by light or air. Although operations were performed in darkness with an aluminum foil jacket, the decomposition ratio of azulene rose to 50 percent during the chromatography.

Azulenic solution (150 ml) was collected (elution volume 600 ml; TP: 260) after evaporating 200 g of mixture, of which 50 percent was azulene. Totals obtained were azulene A, 1160 mg; azulene B, 185 mg.

Thin layer chromatography. Both azulenic solutions were purified by TLC after dilution with ethyl ether to 3 ml for azulene A solution; to 1 ml for azulene B solution. TLC plates of silicagel 60F $_{254}$, with a 2-mm precoated layer

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thickness, were used. The sample size was 500 μl, eluted with hexane, in the absence of light. The blue stripe was extracted (R_f 0.55) with diethyl ether, and the solvent was evaporated to leave a deep blue paste, which was then stored in an opaque bottle under N_2 atmosphere. The following were obtained: 250 mg azulene A, purity 95 percent; 40 mg azulene B, purity 95 percent.

Thus, two blue components were isolated. Although their spectra were identical to that of chamazulene, their boiling points differed, and only component B had the same retention time as chamazulene. Microanalysis gave the following information about the composition of these two species:

azulene A—C% = 92.3; H% = 7.7; $C_{12}H_{12}$
azulene B—C% = 91.2; H% = 8.7; $C_{14}H_{16}$

Only study of the spectra PMR allows the structure of the chamazulene (B) to be confirmed and that of the other azulene (A) to be deduced by comparison.

Spectral analysis

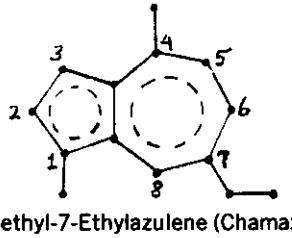
Study of azulene B

1. *UV and visible spectrum.* Compound B showed the following absorption bands: 286—350—370—603—652 mμ. This spectrum is identical to that of 1,4-dimethylazulene except for the 603 mμ band (596 mμ for azulene A).

2. *IR spectrum.* The spectrum shows:
(C-H) at 3080—2950—2920—2870 cm^{-1}
(C=C-C=C) at 1550 cm^{-1}
(C=C-C=C) at 710—690 cm^{-1}

3. *PMR spectrum.**

Bands	Located(ppm)TMS	H	Assignment
triplet	1.83	3	Ar-CH_2-CH_3
quadruplet	2.82	2	Ar-CH_2-CH_3
singlet	2.62	3	1-CH_3
singlet	2.78	3	4-CH_3
doublet	8.04	1	proton 8
doublet	7.50	1	proton 2
2 pairs of doublet	7.21-7.32	1	proton 6 coup. 5
doublet	7.09	1	proton 3 coup. 2
doublet	6.78 and 6.88	1	proton 5 coup. 6



1,4-Dimethyl-7-Ethylazulene (Chamazulene)

These results were confirmed by an Overhauser effect on the 1-methyl; the signal intensity of proton 8 increased by 11.3 percent. These results are also in agreement with the PMR spectrum of chamazulene given by Evdokimoff and coworkers.[6]

Study of azulene A

1. *UV and visible spectrum.* The absorption spectrum of azulene A was identical to that of 1,4-dimethylazulene prepared by Gianotti.[4] It presented absorption bands at: 285—350—367—618—648 mμ.

2. *IR spectrum.* The spectrum showed:
(C-H) at 3020—2960—2940—2850 cm^{-1}
(C=C-C=C) at 1590—1550 cm^{-1}
(C=C-C=C) at 740—775 cm^{-1}

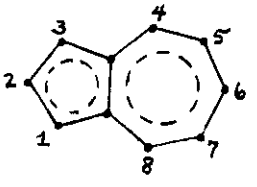
3. *PMR spectrum.**

Bands	Located(ppm)TMS	H	Assignment
singlet	2.6	3	CH_3**
singlet	2.8	3	CH_3
aromatic multiplet	6.8 - 8.2	6	

Attribution of the aromatic multiplet

The chemical shift of azulenic cycle protons follows (/TMS):[5]

4 and 8 : 8.26 ppm
5 and 7 : 7.18 ppm
1 and 3 : 7.33 ppm
2 : 7.82 ppm
6 : 7.51 ppm



Independently of the alkyl substituents, J_{12} and J_{23} are constant and equal to 3.9± 0.2 c/s, while J_1-J_4 decrease with increasing number of alkyl substituents.

With these values, a first interpretation of the aromatic part of the spectrum can be made:

8 or 4 : a doublet located at 8.08 ppm, coupled with 5 or 7, integration : 1 H
2 : a doublet located at 7.50 ppm, coupled with 3, integration : 1 H

On the other hand, irradiation at 8.08 ppm serves to decouple 8 and 7 (in hypothesis of 1,4-dimethylazulene); so the doublet located at 6.9 ppm is attributed to 5 and 7 coupled with 6, and the multiplet located at 7.31 ppm is attributed to 6 coupled with 5 and 7. Both the doublet and the multiplet correspond to an AB_2 spectrum.

*The PMR spectra were run on a Perkin-Elmer R_{32}—90 MHz.

**When a frequency decoupling is applied to the first methyl, an important change of the second doublet in the aromatic multiplet is observed, indicating that the first methyl belongs to the five-carbon cycle of the azulene molecule.

With these results, however, the position of the second methyl could not be determined; it could be either 4 or 8 (fig. 1). A weak irradiation of the 1-methyl was used to detect an Overhauser effect with the possible proton 8, which would be less than 2.5 nm from the 1-methyl, a necessary condition to detect an Overhauser effect. Irradiation at 7.41 Hz (H_2 = 3) of the 1-methyl gave an intensity increase of 10.6 percent, which implies that azulene A is 1,4-dimethylatulene.

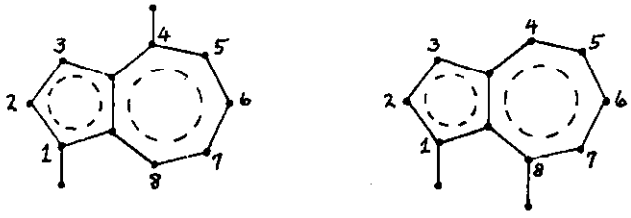


Figure 1. Azulene methyl position. A. 1-4 Dimethyl; B. 1-8 Dimethyl.

Analysis of the total azulene in the essence

The visible spectra of 1,4-dimethylazulene and chamazulene show absorption bands at 596 and 603 mμ. These bands are quite close; the slope of the calibration curves (fig. 2) shows that for chamazulene log ε equals 1.2 x log ε of 1,4-dimethylazulene. At this wavelength only azulenic bands are present. It is therefore possible to make a direct analysis of the total azulenes in the essence. It is not possible to analyze each azulene separately because of the interference of their absorption bands. The analysis is independent of temperature (below 150°) and of time if the products are kept in the dark.

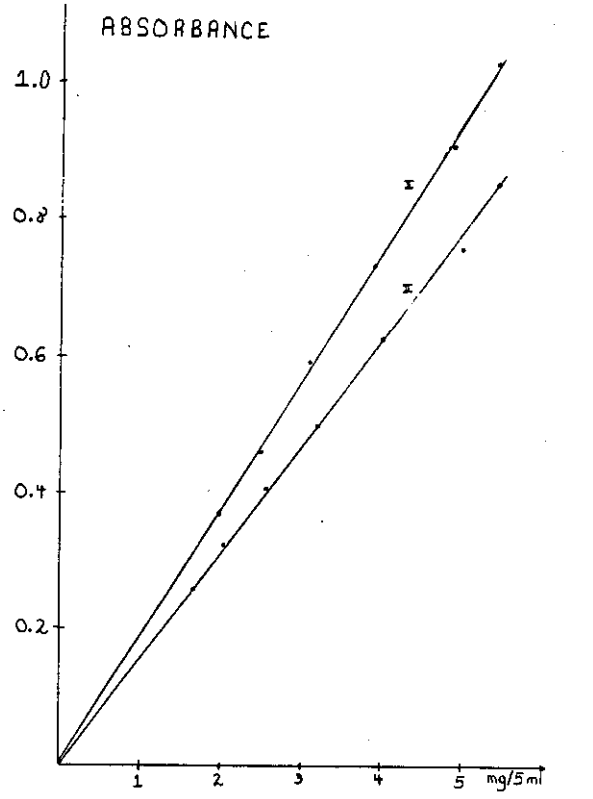


Figure 2. Calibration curves. At 596 mμ I: 1,4 Dimethylazulene. At 603 mμ II: 1,4-Dimethylazulene-7-ethylazulene.

The total percentage of azulene in the essence is 0.38 ± 0.02%. Analysis of the fractions of the distillate described above gave the following percentages in the essence:

0.32 ± 0.02% 1,4-dimethylazulene;
0.07 ± 0.02% chamazulene.

This is in agreement with the percentage obtained in the total analysis.

Conclusion

The study established the nature of the two compounds which produce the deep blue coloration of the essence of *Eriocephalus punctulactus*. These compounds are 1,4-dimethylazulene and 1,4-dimethyl-7-ethylazulene. Their structure is confirmed by the work of Llinas and coworkers on different azulenes, among them 1,4-dimethylazulene, studied by proton and ^{13}CNMR and by computer PMR simulation.[7] Chamazulene is a relatively well-known compound in a number of commercial essences, but 1,4-dimethylazulene has not yet been described in natural essences.

The concentration of azulenes in the essence is 0.32 and 0.07 ± 0.02%, respectively for 1,4-dimethylazulene and for chamazulene. Their presence in the essence does not seem to be a function of their different treatments; thus it can be used as a characteristic datum of the essence. For example, the azulene ratio in different lots of *Eriocephalus punctulactus* essential oil is fairly constant at 0.40%.

Acknowledgment

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