

HIGH RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY IN STUDIES OF PICROTOXANOLIDES OF CORIARIA. CORYAMYRTIN

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RESUMEN

Se discute la evidencia espectroscópica de Resonancia Magnética Nuclear Protónica (400 MHz, y 90 MHz) para coriamirtina, una lactona sesquiterpénica de carácter analéptico, aislada de la planta colombiana *Coriaria microphylla* Poir., mediante analogías con espectros de muestras autenticadas de picrotina y picrotoxina.

SUMMARY

Spectral evidence by Protonic Nuclear Magnetic Resonance (400 MAHz, and 90 MHz) of the analeptic sesquiterpene lactone coriamyrtin, isolated from the colombian plant *Coriaria microphylla* Poir., is discussed, and analogies are made with the spectra of authentic samples of picrotin and picrotoxinin.

Palabras Claves: Resonancia Magnética Nuclear. Coriamirtina.

INTRODUCTION

The plant kingdom has long served as a prolific source of useful medicines for mankind. Every culture in world history has employed

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plant preparations as effective cures for prevalent illnesses without knowledge of the chemistry involved, and primitive remedies have provided the basis for developing modern drugs or leads for researchers to follow. Indeed, this has been so with such compounds that are now known to block nervous transmission as the various δ -aminobutyric acid-antagonists among which picrotoxinin, a picrotoxanolide, is well known. Furthermore, knowledge of primitive uses of plants and their medicinal applications, which has been preserved in the folklore of the South American peoples of the Andes, suggests that convulsant picrotoxanolides could be present in the Andean species of the family Coriariaceae.

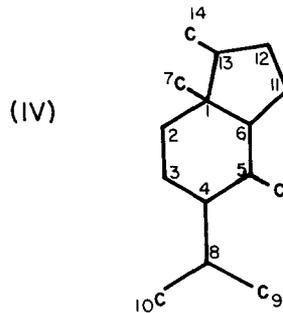
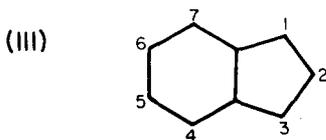
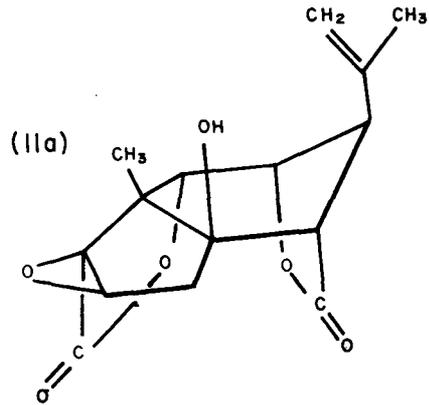
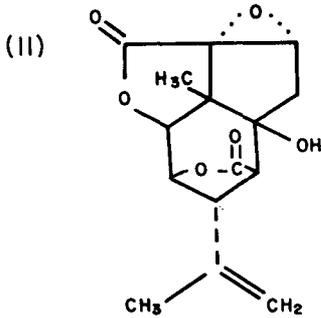
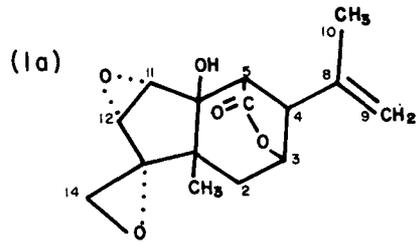
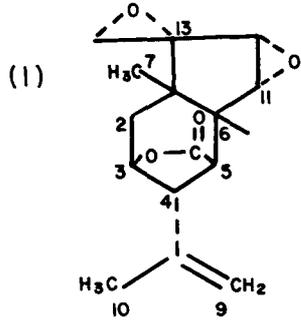
From the above, it was clear that experimental insights into the Colombian species of the family could be most revealing and open new lines of research. Moreover, isolation of pharmacologically-active compounds from species native to areas not yet devoted to intensive agriculture is particularly worthy of study if such species could achieve economic importance.

Although the toxicity of *Coriaria microphylla* Poir. has been known since early colonial times, minimal research has been done on the physiological actions of its extracts or to characterize the active principles. Jaimes de Pino (1972), isolated white needles (mp 228.7°C) which were toxic to mice and dogs and, after infrared spectral analysis of the crystals, she proposed coriamyrtin as the active principle. The author's own research confirms Jaimes de Pino's findings and implies a phylogenetic relationship between the Colombian species and their relatives in other regions of South America.

Coriamyrtin, $C_{15}H_{18}O_5$ (I -IA) is a bitter, toxic sesquiterpene lactone which has also been found in Asian samples of *C. augustissima* Hook (Easterfield and Aston, 1901; Slatter, 1943), *C. lurida* T. Kirk. (Slatter, 1943) and *C. japonica* A. Gray (Kariyone, 1942). More recently Reyes and Ramírez (1980), isolated the compound from the only Chilean species: *C. ruscifolia* L.

The biological properties of coriamyrtin are nearly identical to and parallel exactly those of picrotoxinin $C_{15}H_{16}O_6$ (II -IIa) producing similar, if not identical, symptoms in fish, frogs, mice, rabbits and guinea pigs (Kusumoto, 1933; Shibita, 1955). Both substances counteract barbiturate poisoning (Chen, 1936; Maloney, 1936), but coriamyrtin has not been studied anywhere as thoroughly as picrotoxinin, in regard to its analeptic effects on the central nervous system.

The nomenclature of the picrotoxanolide carbon skeleton has presented difficulties in view of the lengthiness of systematic names.



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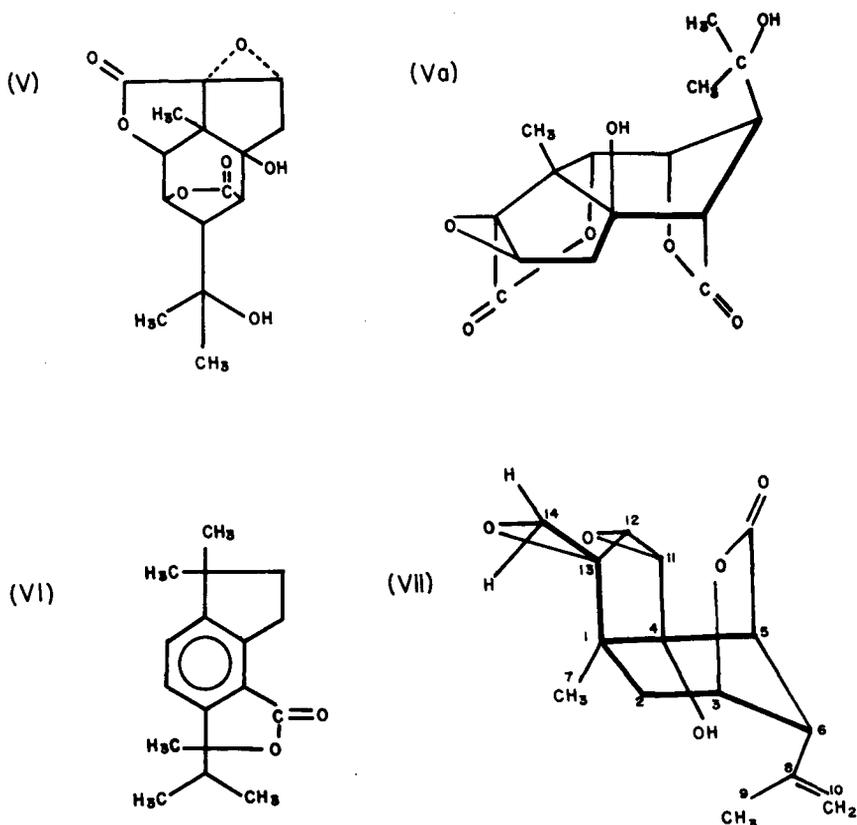
Most modern researchers avoid this by the use of trivial names for the parent compounds and their derivatives and, in fact, the Chemical Abstracts indexes them under the trivial name. More recently, workers of the Japanese Group (Kariyone, 1952; Okuda, 1954; et al.) have based skeletal numbering on the hexahydroindran ring (III), but earlier workers used a numbering system based on a hypothetical, open-chain partial structure devised before the true ring structure was known. Unfortunately, it was confusing and it is preferable to number all structures on the picrotoxane ring as is shown in (IV), a numbering system that is used throughout this work.

Picrotoxinin and picrotin contain two δ -lactones (Hormann, 1916; Conroy, 1952). The presence of a terminal methylene group in the former was determined by bromination (Hormann, 1922), hydrogenation (Mercer and Robertson, 1936; O'Donnel, 1939), ozonization experiments (Harland, 1939), and infrared spectroscopy (Conroy, 1952). Acetylation experiments suggested that picrotoxinin contained one hindered tertiary hydroxyl group and that picrotin contained two, whereas the remaining oxygen atom in both substances was assumed to be in the ether link (Mercer and Robertson, 1936).

Picrotin (V, Va) occurs in plants with picrotoxinin and possesses the same structure except for the addition of the elements of water across the isopropenyl double bond of picrotoxinin, forming a second, tertiary, hydroxyl group. The evidence supporting this relationship was indirect until the same substance was obtained from both compounds (Conroy, 1952). The partial skeleton for picrotoxinin was proposed by Mercer and Robertson (1935), Harlan (1939), whereas Okuda and Yoshida (1964), proposed a structure and absolute configuration for coriamyrtin by analogy with picrotoxinin and the structure of corialactone (VI), itself obtained by aromatization of coriamyrtin (Kariyone and Okuda, 1953). Other experiments, however, indicated a different structure for the compound (Okuda, 1964) and, although the evidence provided by ^1H NMR indicated that the five-membered ring has not been opened, it was based on 30 MHz. It appears that high resolution ^1H NMR (400 MHz) of the compound isolated from colombian *C. microphylla*, combined with evidence provided by the application of the same techniques to the parent compounds, (picrotoxinin and picrotin), should yield data on the absolute configuration of a substance which is toxic to man and animals and undoubtedly merits thorough ethnobotanical and phytochemical attention. The aim of this paper is to provide such evidence.

EXPERIMENTAL

Voucher specimens were deposited in the Herbario Nacional Colombiano, Instituto de Ciencias Naturales, Universidad Nacional de



Colombia (López-140; Aguirre & López 1908, López 141). The collection site was the outskirts of Usme (Special District of Bogota) at an elevation of 3.000 - 3.5000 meters. Duplicates of specimens were studied and identified by the author at the Herbarium of the Royal Botanic Gardens Kew, Surrey, England, where comparisons were made with world-wide collections of *Coriaria* sp. The aerial part of the dried, ground plant (4 Kg) was subjected to percolation with hot aqueous methanol (40%). After purification of the extract (CC, TLC, fractional crystallization), 997 mg of colourless, bitter, prisms were obtained.

Melting point determinations were made in a Buschi-SMP20 apparatus. All spectral analyses were carried out on the prisms and on picrotin and picrotoxinin for comparison. IR measurements were taken in a double beam Perking-Elmer 237 Grating Infrared spectrophotometer fitted with a thermocouple detector after dispersion in Nujol-mull.

Proton nuclear magnetic resonance spectra at 400 MHz, were obtained through the ULIRS WH-400 NMR Service of the University of London at Queen Mary College, along with expansions, integrations and D₂O - exchange. 90MHz - ¹H NMR was carried out in a Perking-Elmer R-32 spectrometer fitted with and S51B oscilloscope, magnetic field intensity of 21.140 gauss and decoupling strength to 8 miligauss on field and frequency modes. For comparison, ¹H NMR spectra of authenticated samples of picrotoxinin and picrotin were obtained in several solvents after the spectra of those solvents in pure solution had been determined to record their resonance absorptions.

Once these parameters were established, sufficient amounts of the crystals isolated from the plant material were dissolved in ca 0.5 ml of these solvents to give a concentration of about 80-90 mg/ml, using TMS as internal reference and locking the entire scan to the internal reference peak located at 0 ppm (sweep range 10 ppm and filter 2). After obtaining the resonance absorptions, the signals were integrated and expansions were carried out at a sweep range of 300 Hz.

Solvents used were:

- a. Deuteriochloroform, which gives a resonance peak at 7.2 ppm because of the small amounts of protochloroform present. This solvent allows observation of hydroxyl signals in the spectra, and addition of a few drops of deuterium oxide usually results in rapid exchange of the hydroxylic protons which is sufficient to remove those peaks. However, the compounds studied are not readily soluble in chloroform.
- b. Pyridine causes changes in chemical shifts and, because coupling constants are not affected by the nature of the solvent, provides a basis for the study of overlapping bands in CDCl₃. The solvent absorbs strongly between 4.5 and 10.0 ppm but, since the compounds under study do not absorb in the aromatic region, peaks caused by pyridine do not interfere with those of the crystals. Nevertheless, the hydroxyl signals of the samples do not show up in pyridine as this solvent is hydrogen-bond, and therefore, D₂O exchange is not possible.
- c. Dimethylsulphoxide -d₆ was used throughout, because it shifts the hydroxyl resonance downfield and, thus, allows observation of protons attached to oxygen. Chapman (1964) found that, in dimethylsulphoxide solutions, strong hydrogen bonding reduces the rate of proton exchange sufficiently to permit observation of hydroxyl proton splitting, and that these resonances are below the C¹³ side-band of DMSO. This is so because alcohols are often sparingly-soluble in the common NMR solvents, and their hydroxyl

resonances are obscured by methyl and methylene absorptions; traces of acid always present in these solvents catalyze the proton exchange so that spin-spin splitting of hydroxyl peaks is rarely observed.

Thus, DMSO may be employed to study hydroxylic protons since primary, secondary and tertiary alcohols give clearly resolved triplets, doublets, and singlets, respectively (McGreer and Moceck, 1963). Addition of a few drops of deuterium oxide is sufficient to remove the hydroxyl hydrogens because their exchange in DMSO is slow relative to the nuclear spin inversion but is still sufficiently rapid to permit complete exchange of hydroxyl protons for deuterons in a few minutes. Therefore, signals that are due to hydroxyl groups may be identified even when superimposed on another multiplet. Furthermore, resonances of the OH groups of the compounds under investigation were always below 5.8 ppm and did not overlap with any other signal.

RESULTS AND DISCUSSION

Coriamyrtin (I), prisms (m.p. 228.5°C); Rf 0.13 (chloroform/silica gel); IR $\nu_{\text{max}}^{\text{Nujol}}$ 3.600-3.200 (OH) 1770, 1760 (lactone), 1650 (C=C), 1160 cm^{-1} (epoxide).

90 and 400 MHz - ^1H NMR Spectroscopy

The 400 MHz spectrum of coriamyrtin in DMSO (Table 1 and Figure 1) reveals a singlet at 1.00 ppm which is equivalent to three protons and may be attributed to a C-7 angular methyl group. At 90 MHz, in the same solvent, the signal is located at 1.01 ppm and also integrates three protons (Figure 2). The assignment of this singlet to a C-7 methyl group is in agreement with the one made by Okuda and Yoshida (1967), and with the angular methyl signal exhibited by picrotoxinin and picrotin. Both compounds possess an angular methyl group. The resonance of the C-7 methyl absorption in the 400 MHz spectrum in CDCl_3 is 1.20 ppm, and in pyridine the peak is located at 1.35 ppm at 90 MHz (Table 1). The three protons of the terminal methyl group of the isopropenyl moiety can be detected in the various solvents as follows: At 400 MHz 1.88 ppm in DMSO and 1.62 ppm in CDCl_3 , whereas at 90 MHz the signal is centered at 2.01 ppm in pyridine, 1.93 ppm in CDCl_3 and 1.90 ppm in DMSO.

The integration for this signal is three protons in all spectra. This absorption agrees with that of picrotoxinin, which contains an isopropenyl moiety, but the peaks are located a few ppm upfield, probably because of the absence of a free hydroxyl group at C-2.

TABLE 1

¹H NMR assignments of coriamyrtin in various solvents

| Signal (ppm) | CDCl ₃ | | PYRIDINE | DMSO | | Integration |
|--|--------------------|---------------------|--------------------|--------------------|---------------------|-------------|
| | 90 MH _z | 400 MH _z | 90 MH _z | 90 MH _z | 400 MH _z | |
| C-7 angular methyl | 1.12 | 1.20 | 1.35 | 1.01 | 1.00 | 3 |
| C-10 methyl group | 1.92 | 1.62 | 2.01 | 1.90 | 1.88 | 3 |
| C-9 | 4.63 | 4.69 (J=2.3) | 4.62 | 4.58 | 4.60 | 2 |
| Terminal epoxide C-14 (AB quartet) | 2.85 3.25 | 3.07 3.28 | 2.93 3.28 | 2.75 3.15 | 2.84 3.28 | 2 |
| C-OH | 2.35 | 1.60 | — | 5.4 | 5.68 | 1 |
| C-2 | 1.70 | 1.88 | 1.75 | 1.65 | 1.56 | 2 |
| C-3 | 4.8 | — | 4.8 | 4.9 | — | 1 |
| C-11 | 4.93 | 4.98 | 4.88 | 4.82 | 4.91 | 1 |
| C-12 | 4.93 | — | 4.88 | 4.82 | — | 1 |
| C-4 | 2.80 (J=5.2) | 3.07 | 2.93 | 2.78 | 2.84 | 1 |
| C-5 | 3.72 | 3.75 (J=3.2) | 3.88 | 3.65 | 3.70 | 1 |

The methylenic part of the isopropenyl group is located far downfield (Table 1) as a diffused doublet at 4.63 ppm in CDCl₃, 4.58 ppm in DMSO and 4.62 ppm in pyridine, all at 90 MH_z. At 400 MH_z, this signal appears at 4.69 ppm in CDCl₃, and at 4.60 ppm in DMSO (Figures 1 and 4). This diffused doublet is fairly well resolved at 400 MH_z, as a doublet with a coupling constant of J=2.3 cps, and is

equivalent to two protons (peaks 1 and 2, Table 2). Irradiation of the singlet at 1.92 ppm, which is due to the presence of the C-10 methyl group, shows that a slight coupling occurs between the two constituents of the isopropenyl group in the molecule, and further supports the assignments made on these two absorptions.

Picrotoxinin presents a similar pattern of resonance as regards its isopropenyl group.

TABLE 2

Expansions in the ^1H -400 MHz, spectrum of coriamyrtin in CDCl_3 ,

| Peak | Frequency (cps) | Chemical Shift (ppm) | Integration | Intensity |
|------|-----------------|----------------------|-------------|-----------|
| 1 | 1870.669 | 4.675 | 0.306 | 5.816 |
| 2 | 1868.321 | 4.669 | 0.299 | 5.703 |
| 3 | 1312.549 | 3.280 | 0.03 | 3.748 |
| 4 | 1309.321 | 3.272 | 0.03 | 4.126 |
| 5 | 1228.626 | 3.070 | 0.05 | 6.326 |
| 6 | 1225.398 | 3.062 | 0.07 | 7.343 |
| 7 | 1928.770 | 4.820 | 0.31 | 1.957 |
| 8 | 1927.596 | 4.817 | 0.74 | 3.868 |
| 9 | 1926.422 | 4.814 | 0.55 | 2.807 |
| 10 | 1924.368 | 4.809 | 0.061 | 3.286 |
| 11 | 1923.194 | 4.806 | 0.186 | 5.806 |
| 12 | 1922.021 | 4.803 | 0.083 | 3.522 |
| 13 | 1919.966 | 4.798 | 0.049 | 3.092 |
| 14 | 1918.793 | 4.795 | 0.087 | 4.116 |
| 15 | 1917.619 | 4.792 | 0.041 | 2.599 |
| 16 | 1234.788 | 3.086 | 0.002 | 4.791 |
| 17 | 1229.506 | 3.073 | 0.006 | 6.070 |
| 18 | 1224.224 | 3.059 | 0.008 | 6.940 |
| 19 | 1507.392 | 3.767 | 0.258 | 14.972 |
| 20 | 1504.164 | 3.759 | 0.276 | 15.374 |

A C-10 methyl peak is exhibited at 1.90 ppm and a doublet, equivalent to the C-9 ethylene absorption, at 4.85 ppm in CDCl_3 . This pattern supports the assignments made of the compound studied.

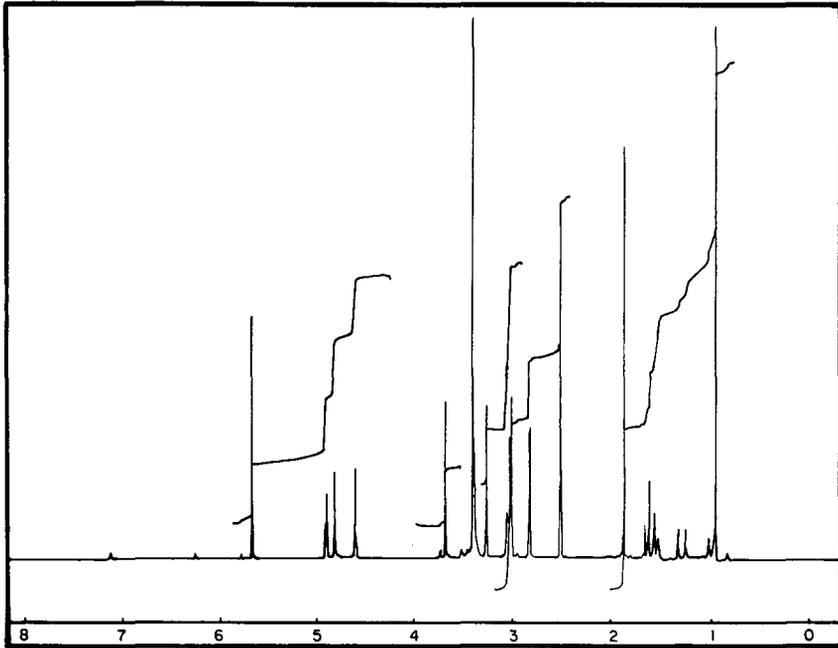


Fig. 1- ¹H-400 MHz NMR Spectrum of Coriamyrtin in DMSO.

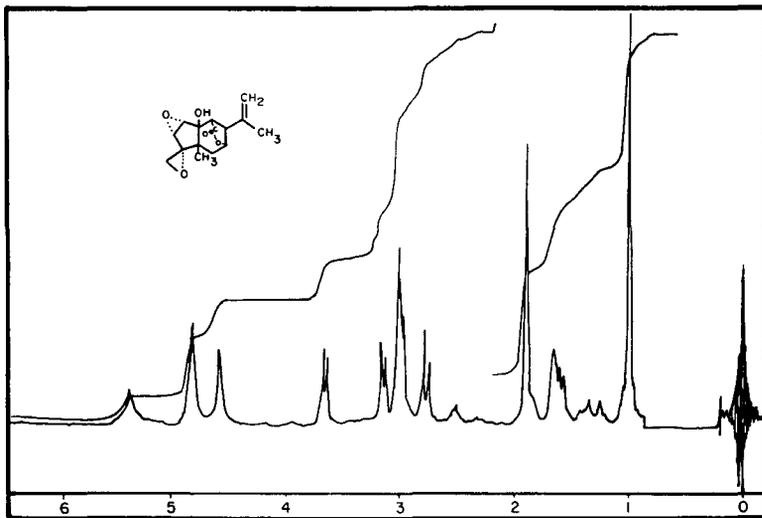


Fig. 2 ¹H-90 MHz NMR Spectrum of Coriamyrtin in DMSO.

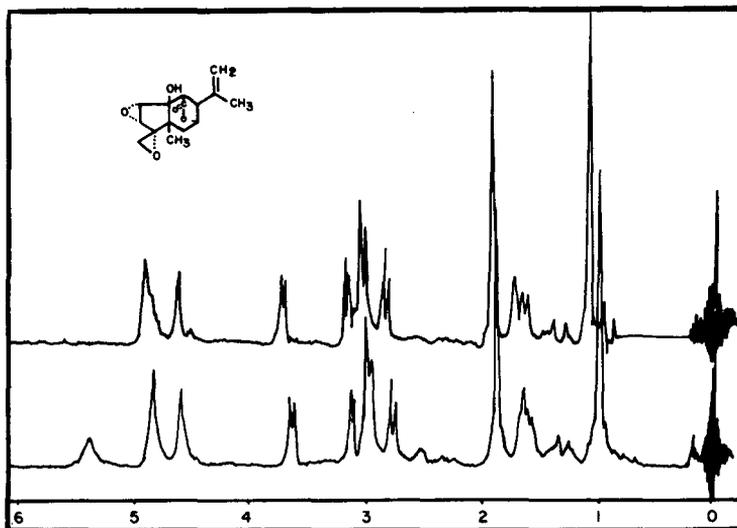


Fig. 3 ¹H-90 MHz NMR Spectrum of Coriamyrtin in DMSO.
after D₂O Exchange.

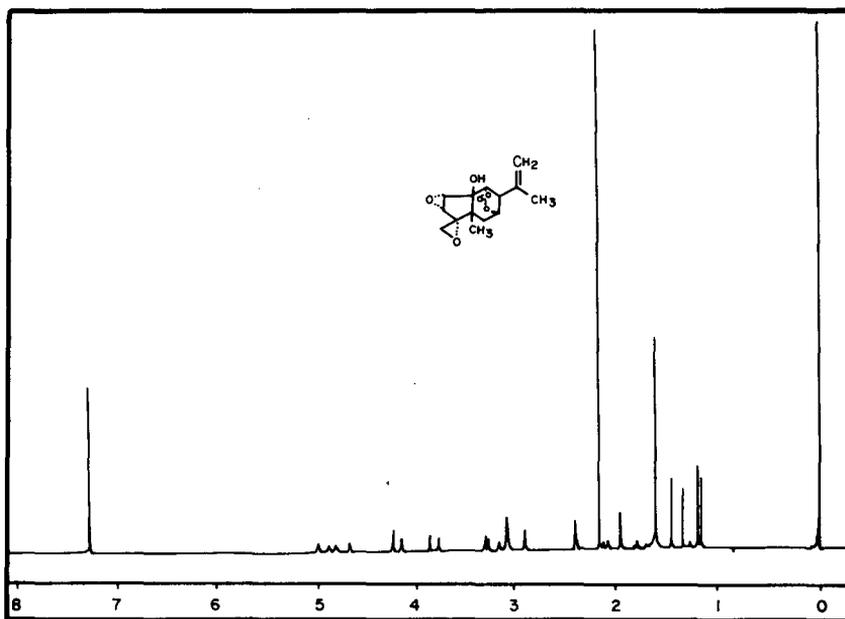


Fig. 4 - ¹H-400 MHz NMR Spectrum of Coriamyrtin in CDCl₃.

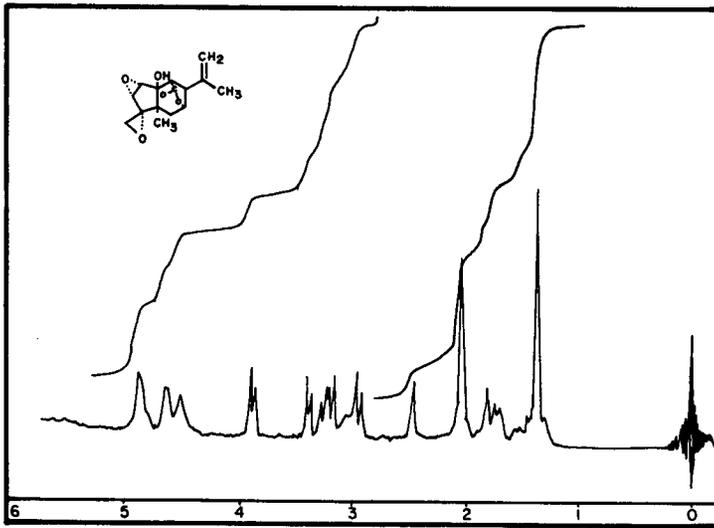


Fig. 5 - ¹H-90 MHR Spectrum of Coriamyrtin in Pyridine.

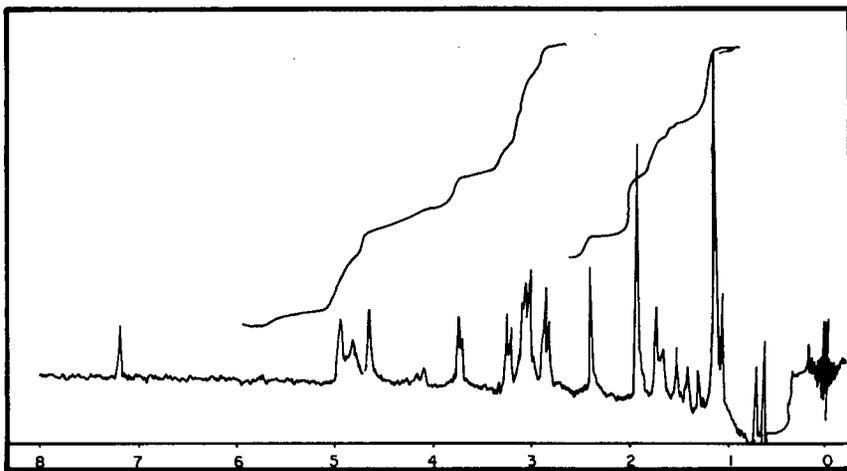


Fig. 6 - ¹H-90 MHz NMR Spectrum of Coriamyrtin in CDCl₃.

The terminal epoxide protons can be seen as an AB quartet, whose locations are as follows: At 90MHz, doublets at 2.85 and 3.25 ppm in CDCl₃; 2.75 and 3.15 ppm in DMSO; 2.93 and 3.28 ppm in pyridine; at 400 MHz, doublets at 3.07 and 3.28 ppm in CDCl₃, and at 2.84 and 3.28 ppm in DMSO. The coupling constant is (Figure 7):

$$\Delta 3 - 4 = 1312.549 - 1309.321 = 3.228$$

$$\Delta 5 - 6 = 1228.626 - 1225.398 = 3.228$$

$$\Delta v = \sqrt{(83.923)^2 - (3.228)^2} = 83.8608$$

$$JAB = \sqrt{(83.923)^2 - (83.8608)^2} = 3.23 \text{ cps}$$

Only one hydroxylic proton occurs in the molecule, as is seen in the 400 and 90 MHz spectra of the compound in DMSO, whereas the chemical shift of this proton in CDCl₃ is 2.35 ppm at 90 MHz and 1.60 ppm at 400 MHz. All spectra show that the resonance assigned to the C-6 OH integrates for one proton and that addition of D₂O increases its exchange rate, making the signal disappear a few minutes in CDCl₃, and in DMSO (Figure 1). Thus, the compound under investigation has only one hydroxyl group, which is located at position C-6 as the peaks in the various solvents are always singlets, and therefore the hydroxyl substituent must be attached to a tertiary carbon atom. It will be shown that the other two tertiary carbon atoms available for substitution by a hydroxyl group, C-5 and C-4, are occupied by protons with splitting patterns that do not permit attachment of a hydroxyl group at this part of the molecule. Furthermore, the C-6 hydroxylic substitution is also exhibited by the spectra of both picrotin and picrotoxinin at the same chemical shift that has been assigned to the hydroxyl group of coriamyrtin.

The two protons on C-2 resonate as diffused doublets located at 1.70 ppm in CDCl₃, 1.65 ppm in DMSO, and 1.75 ppm in pyridine (90 MHz). The spectrum at 400 MHz shows these absorptions as doublets centered at 1.88 ppm in CDCl₃. These signals integrated for two protons. The splitting patterns exhibited by the spectrum in DMSO at 400 MHz, where the signal from the C-2 protons occurs at 1.56 ppm, shows a multiplet made of seven peaks. This splitting suggests that the two protons might be coupled to both the C-3 protons and the angular methyl group, although the latter coupling is not strong enough to be detected by the spectra taken at 90 MHz, and only coupling to the C-3 hydrogens is shown. Frequency-decoupling experiments showed that irradiation of the band attributable to the C-3 proton brings about changes in the diffused doublets of the two C-2 protons whereby they become a clear doublet. Conversely, irradiation of the C-3 protons collapses one of the peaks which constitute the C-3 band, and thus two

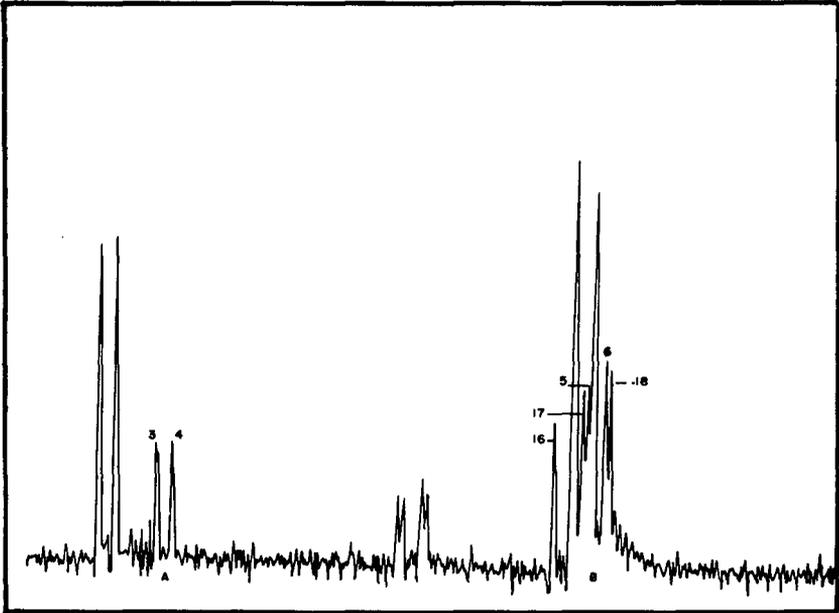


Fig. 7 - Expansions of the ^1H -400 MHz Spectrum of Coriamyrtin in CDCl_3 .

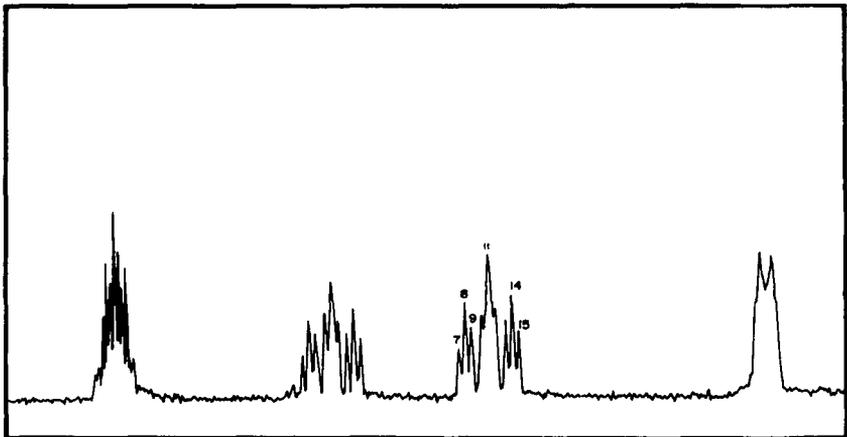


Fig. 8 - Expansions of the ^1H -400 MHz NMR Spectrum of Coriamyrtin in CDCl_3 .

peaks are visible. These couplings are also confirmed by field decoupling experiments at frequencies of + 310 and - 310 cps. As mentioned above, the band assignable to the C-3 proton is coupled to the protons positioned at C-2. This band has a chemical shift at 4.8 ppm in CDCl_3 (90 MHz) and is superimposed on other peaks arising from the epoxide protons at C-11 and C-12 in DMSO and pyridine, at 4.9 and 4.8 ppm, respectively.

The 400 MHz spectrum in CDCl_3 exhibits a C-3 triple triplet at 4.80 ppm, whereas in DMSO a triplet occurs at 4.90 ppm. (Figures 1 and 4).

The coupling constants are (Table 2 and Figure 8):

$$\begin{aligned}\text{Center of first triplet} &= 1/2 (1928.770 + 1926.422) = 1927.596 \\ \text{Center of second triplet} &= 1/2 (1924.368 + 1922.202) = 1923.1945 \\ \text{Center of third triplet} &= 1/2 (1919.966 + 1917.619) = 1918.7925\end{aligned}$$

$$\text{JAB} = 1927.596 - 1923.1945 = 4.402 \text{ cps}$$

$$\text{JBC} = 1923.1945 - 1918.7925 = 4.402 \text{ cps}$$

$$\text{JAC} = 1927.596 - 1918.7925 = 8.80 \text{ cps}$$

The hydrogens attached to C-11 and C-12 in the epoxide system exhibit resonances at the following chemical shifts (Table 1): At 90 MHz : 4.82 ppm in DMSO; 4.93 ppm in CDCl_3 , and 4.88 ppm in pyridine (Figures 3, 6 and 5, respectively).

These signals consist of a broad band equivalent to three protons, two of which belong to the epoxide ring and a third to the C-3 proton. At 400 MHz the latter proton appears as a separate triple triplet, leaving a multiplet assignable to the epoxide protons at 4.98 ppm in CDCl_3 and a triplet located at 4.91 ppm in DMSO (Figures 1 and 4).

Irradiation did not show a significant coupling of the epoxide protons to any other signal.

The doublet at 2.80 ppm in CDCl_3 (90 MHz) integrates for one proton, and can be attributed to the hydrogen at C-4 (Figure 6). A similar doublet is seen in DMSO (90 MHz) located at 2.78 ppm (Figure 3) and at 2.93 ppm in pyridine (Figure 5), whereas the 400 MHz spectra demonstrates the C-4 proton to resonate at 2.84 ppm in DMSO and 3.07 ppm in CDCl_3 , which can be seen as a triplet superimposed on the second half of the AB quartet arising from one of the terminal epoxide protons at C-14. The peaks of the triplet (Figure 7) are separated by 5.28 cps (Table 2).

The coupling constant is:

$$\Delta 17-18 = \Delta 16 - 17 = 1234.788 - 1229.506 = 5.282 \text{ cps}$$

$$JAB = 5.28 \text{ cps}$$

$$JAC = 10.56 \text{ cps}$$

Frequency and field-decoupling experiments indicate that the C-4 triplet is coupled to the C-5 proton, as irradiation of one signal causes the other to collapse, and conversely. The proton on C-5 gives a doublet, equivalent to one proton, located at 3.72 ppm in CDCl_3 , 3.65 ppm in DMSO, and 3.88 ppm in pyridine (90 MHz). At 400 MHz , the proton resonates at 3.75 ppm in CDCl_3 and at 3.70 ppm in DMSO. The coupling constant is (Table 2 and Figure 9):

$$JAB = 1507.392 - 1504.164 = 3.22 \text{ cps}$$

That the signal is a doublet, suggests that it is not coupled to the C-6 hydroxyl group and supports the assumption of a quasichair conformation of the molecule (VII), where the two groups are moderately separated from each other. Decoupling experiments, on the other hand, confirm this assumption and also show that the C-5 proton is coupled to the C-4 signal.

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