COMPUTER-ASSISTED STRUCTURE ELUCIDATION OF HUMULENE EPOXIDE AND CARYOPHYLLENE EPOXIDE MIXTURE OF *TURRAEA BROWNII*

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ABSTRACT

An experiment was carried out to isolate active material from Turraea brownii for biological insectiside. The isolated component obtained by using TLC was elucidated with ^1H NMR, and ^{13}C NMR techniques. It was found that the compound was unknown triterpenoid ($C_{30}H_{48}O$). Further elucidation using 2D NMR with COSY, HMBC, and HMQC combined with computer techniques showed that the component was an approximately equimolar mixture of two sesquiterpenes ($C_{15}H_{24}O$) which were identified as humulene epoxide and β-caryophyllene epoxide.

INTISARI

Suatu percobaan telah dilakukan untuk mengisolasi bahan aktif dari Turraea brownii untuk insektisida biologi. Struktur kimia hasil isolasi dengan teknik kromatografi lapisan tipis ditentukan menggunakan ^1H NMR dan ^{13}C NMR. Hasil sementara menunjukkan bahwa senyawa tersebut adalah triterpenoid ($C_{30}H_{48}O$) tak dikenal. Penentuan struktur kimia selanjutnya menggunakan 2D NMR dengan teknik COSY, HMBC dan HMQC serta didukung program komputer. Ternyata komponen tersebut merupakan campuran sebanding dari sesquiterpenoid ($C_{15}H_{24}O$) yang telah diidentifikasi sebagai epoksida humulena dan epoksida β-karyofilena.

INTRODUCTION

Structure elucidation is a difficult task and in all cases, this is a time consuming operation. However the problem can be overcome by the availability of analytical instruments and their operators since various techniques are now well-known for structure determination, i.e.; x-ray crystallography, mass spectrometry, infra-red, ultraviolet, circular dichroism, nuclear magnetic resonance (NMR), elemental analysis and the classical chemistry. Until now, NMR is still the most powerful tool for structure determination (1).

The use of NMR in structure elucidation has grown unabated in power and versatility, conspiciously since the late 1970s with the introduction of Fourier Transform (FT) NMR spectrometry (2). Fortunately, the technique can still be applied to most problems of structure determination without the help of experts. With FT-NMR it is possible to carry out modified experiments such as; spin decoupling, Nuclear Overhouse Enhancement (NOE) and Correlated Spectroscopy (COSY) (3,4). These experiments are useful to assign structures which are difficult to solve by conventional method. Recently, Dr. J.M. Nuzillard of the University of Reims in France, has developed a computer programme which is capable of using data for HMQC (Heteronuclear Multiple Quantum Correlation) and HMBC (Heteronuclear Multiple Bond Correlation) for two or three bond C-H connectivities spectra to assign structures automatically (1), as shown in Figure 1.

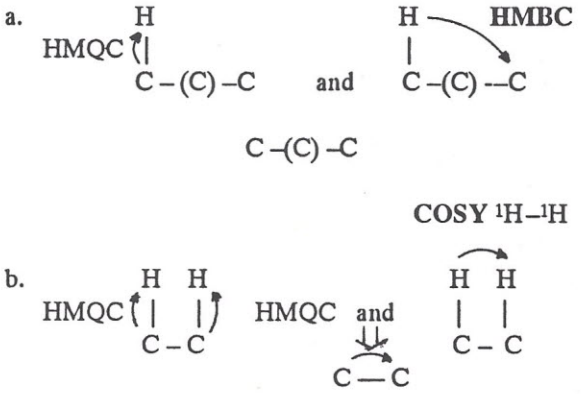


Figure 1. Combination of HMBC, HMQC and COSY spectra to yield C-C inferred correlations.

These techniques were used to elucidate and conform the structure of triterpenoid isolated from *Turraea brownii*, as described in this article.

EXPERIMENTAL METHODS

Material and General Procedures

All solvents used in the extraction and chromatographic separations were distilled. Petroleum ether refers to the fraction of boiling point 40°-60°C. Silica gel 60 GF_{254} was used in TLC and flash column chromatography.

^1H NMR measurements were made in $CDCl_3$ solution, on Bruker WP200SY instrument with TMS as an internal standard at δ 0 or $CHCl_3$ at δ 7.25 and all signal are reported as δ values. ^{13}C NMR spectra were obtained at 50 M Hz with $CDCl_3$ as an internal standard and solvent at

δ 77.0. The chemical shifts are reported in ppm downfield from TMS. All 2D spectra have been recorded on a Bruker-AC 300 instrument. The HMBC and HMQC data were set at 256 FIDs of 2 K points. Direct and long-range coupling constants have been choosen on average equal to 135 Hz and 7 Hz respectively. Mass spectra were determined on A.E.I.-G.E.C. MS-12 mass spectrometer, high resolution spectra being obtained on an A.E.I.-MS-902S instrument. Mass spectra were also obtained with a Hewlet-Packard 5880-A (FID detector) combined with a Varian 1400 chromatograph coupled to a Varian MAT CH-7A spectrometer, using column CP sil 5 CB (25 m x 0.32 mm x 0.12 m).

Flash column chromatography was conducted on Merck silica gel 60 GF_{254} (70-230 mesh). Preparative TLC utilized silica gel GF_{254} coated to 1 mm thickness and viewed under UV light or by spraying with 25% sulfuric acid followed by heating (110°C, 5 min).

Experiment

The dried ground stem bark (426.37 g) was extracted by exhaustive percolation with ethyl acetate. Evaporation of the ethyl acetate extract under pressure, left a dark green oily residue (16.13 g) which was extracted with ether. Evaporation of the ether gave a dark green residue (11.93 g) which was subjected to chromatography on silica gel dry column (100 g). Elution was carried out using mixtures of petroleum ether, ethyl acetate and methanol of increasing polarity. A total of 38 fractions were combined into groups according to the similarity of their TLC patterns on silica gel GF_{254} using mixture of petroleum ether and ethyl acetate.

Replating of fraction 10 (F10) on preparative plates using petroleum ether - ethyl acetate (98 - 2) as the solvent gave a strongly UV active band (F10B4) as the major component. This band was removed and extracted with ethyl acetate to yield F10B4 as a colourless residue (15.4 mg).

RESULTS AND DISCUSSION

The product isolated from fraction 10 (F10) by preparative TLC using petroleum ether-ethyl acetate (98 - 2) gave very interesting NMR spectra. Its ^1H NMR spectrum (7) exhibited seven methyl (δ_H 1.54) and six quaternary methyl [δ_H 0.96, 0.98, 1.05, 1.08, 1.17 and 1.28 - the chemical shifts of the two quaternary methyl at δ_H 1.17 and 1.28 suggests their being attached to a carbon linked to oxygen (5)], a vinyl group system at δ_H 5.1 - 5.4, an exocyclic methylene at 4.86 and 4.97, and two one proton signals centered at δ_H 2.87 (dd, J 8 Hz) and 2.54 (dd, J 8.4 Hz) arising from trisubstituted epoxides. Its ^{13}C DEPT NMR spectrum (7) showed that there are 30 carbon atoms consisting of 7 methyls, 10 methylenes, 7 methines and 6 non protonated carbons. The assignment of the fuctionality can be seen in Table 1.

Table 1. ^{13}C NMR assignment of compound F10B4.

Chemical shifts (ppm)	Functionality
15.0, 16.9, 17.1, 21.6, 27.2	methyl groups
59.8, 61.9, 63.2, 63.7	epoxides
112.7, 122.0, 125.7, 131.8, 143.0, 151.7	double bonds

It was difficult to relate the above data to known triterpenoid system and it was then examined with 2D NMR. Proton-proton COSY and direct $^{13}C/^1H$ two dimensional chemical shift correlation (HMQC) experiment were run (7) and led to the assignment of some part structures as shown below.

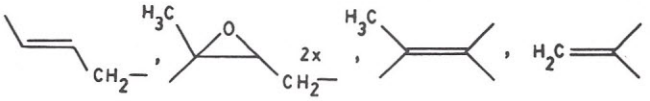


Figure 2. Part structures of F10B4.

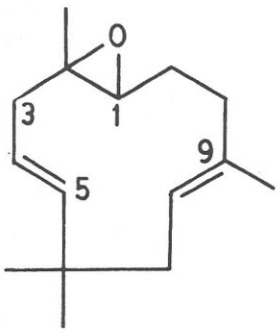
Clearly more connectivity was required and so a long range $^{13}C/^1H$ experiment (HMBC) was run using inverse detection which greatly increases the sensitivity of the experiment. Many correlations were observed, so complicated that it was very difficult to assimilate the data and use them for derivation of a rational structure. It was decided to use a computer programme which is developed by Dr. J. M. Nuzillard for F10B4.

Table 2. ^{13}C NMR chemical shifts of humulene epoxide and caryophyllene epoxide in $CDCl_3$.

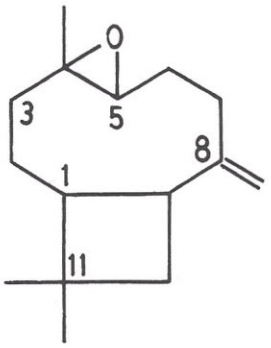
Carbon	Humulene epoxide		Caryophyllene epoxide	
	δ_C (mult)E*	δ_C (mult)R*	δ_C (mult)E*	δ_C (mult)R*
C-1	61.87 d	61.87 d	50.68 d	50.95 d
C-2	63.17 s	63.11 s	30.15 t	30.29 t
C-3	42.53 t	42.70 t	39.10 t	39.32 t
C-4	122.04 d	122.25 d	59.76 s	59.67 s
C-5	143.05 d	143.12 d	63.69 d	63.69 d
C-6	36.21 s	36.53 s	29.51 t	29.96 t
C-7	40.53 t	40.36 t	29.73 t	30.09 t
C-8	125.66 d	125.83 d	151.76 s	151.89 s
C-9	131.84 s	131.94 s	48.68 d	48.87 d
C-10	36.57 t	36.78 t	39.70 t	39.91 t
C-11	24.69 t	24.89 t	33.96 s	34.06 s
C-12	17.15 q	17.29 q	16.95 q	17.03 q
C-13	25.48 q	25.67 q	27.15 q	27.70 q
C-14	28.97 q	29.05 q	21.58 q	21.90 q
C-15	15.03 q	15.01 q	112.70 t	112.83 t

Note: E* - Experimental. R* - Reference (6). mult - multiplicity. s - singlet. d - doublet. t - triplet. q - quartet.

Since there were some overlaps in the ^{1}H NMR spectra, it will only clearly defined by correlations in which the HMQC and HMBC were used. Surprisingly, these correlation techniques produced results which indicated that the compound consisted of a mixture of humulene epoxide and caryophyllene epoxide. Comparison with the ^{13}C chemical shifts of humulene epoxide and ß-caryophyllene epoxide (see Table 2) confirmed that F10B4 was indeed a mixture of the two compounds. GC-MS analysis revealed the components of the mixture with retention times of 14.45 and 15.02 minutes, respectively.



Humulene epoxide



ß-Caryophyllene epoxide

CONCLUSION

This experiment represents a particularly impressive example of the capability of computer assisted structural elucidation. The computer lacks the prejudices of a human and remains uninfluenced by biogenetic consideration. It does not require to know whether long range correlation between carbons and protons are $^{2}J_{CH}$ or $^{3}J_{CH}$ since the programme considers all possibilities. There is little doubt that this computer approach to structural elucidation will find wide application once the advantages and the possibilities are realised.

ACKNOWLEDGEMENTS

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REFERENCES

1. J.M. Nuzillard and G. Massiot, Computer - assisted Elucidation of Structure of Natural Products, *Phytochemical Analysis,* 1992, 3, 153.
2. D. Shaw, Fourier Transform NMR Spectroscopy, 2nd ed.,Elsevier, Amsterdam, 1984.
3. D.H. Williams and I. Fleming, Spectroscopic Methods in Organic Chemistry, 4th ed., McGraw-Hill Book Company, London, 1989.
4. J.K.M. Saunders and B.K. Hunter, Modern NMR Spectroscopy, OUP, Oxford, 1987.
5. D.A. Mulholland and D.A.H. Taylor, Limonoids From The Seed *of Entandrophragma caudatum, Phytochemistry,* 1988, 27, 1220.
6. N.P. Damodaran and S. Dev, Stereochemistry of Zerumbone, *Tetrahedron Letters*, 1965, 24, 1977.
7. Tahid, Modern NMR Methods in the Structural Elucidation of Natural Product, M.Sc. Thesis, Chemistry Department, University of Glasgow, 1993.