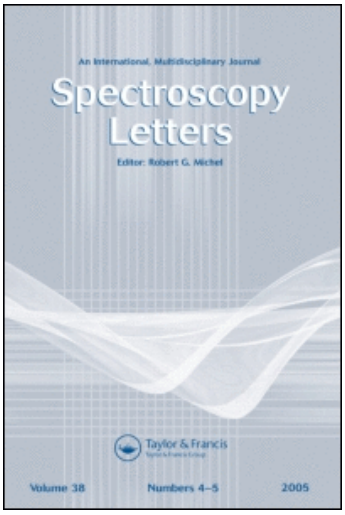

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PMR ASSAY OF ESSENTIAL OILS : V Assay of Benzyl Benzoate and Benzyl Cinnamate in Balsams.

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Keywords: Benzyl benzoate, NMR Analysis; Benzyl Cinnamate, NMR Analysis; NMR Analysis, Balsam of Peru; NMR Analysis, Balsam of Tolu; NMR Analysis, Tolu balsam; NMR Analysis, Peru balsam.

Abstract

An accurate, simple and precise PMR procedure has been developed for the quantitation of benzyl benzoate (I) and benzyl cinnamate (II) as pure drugs and in Peru and Tolu balsams. The average recovery of pure I and II in standard mixtures using ethyl benzoate (III) as the internal standard was 100.2± 0.38% w/w and 100.0± 0.34 34% w/w respectively. The results of the assay using standard synthetic mixtures containing both I and II proved to be reproducible and gave a mean of 100.0 ± 0.54 w/w for I and 100.0 ± 0.62 w/w for II. Applying the same method for the determination of I and II in balsams, an average

value of 44.7% w/w was obtained for I and 4.4% w/w for II in Peru balsam. The corresponding average values for Tolu balsam were 11.6 w/w and 14.3% w/w. Moreover the method provides means for the ratio determination of I and II and the identification of the balsams.

Introduction

Balsams are resinous mixtures which contain large proportions of benzoic and/or cinnamic acids and esters of these acids (1). Peru and Tolu balsams are obtained from *Myroxylon pereina* (Royle) Klotzsch and *Myroxylon balsamum* (Linn) Harms, respectively (Fam. Leguminosae). Both balsams are used as antiseptics and expectorants and in perfurmary. Peru balsam is currently used as an ingredient of dental cements. Tolu balsam is also used as a flavouring agent in medicinal syrups and in confectionary (2,3). The total ester content of Peru balsam varies from 47-66% (4-6). Tolu balsam has been reported to contain about 7% of a volatile oil composed chiefly of I, II, ethyl benzoate and ethyl innamate (5).

The determination of the ester contents is of great importance in the evaluation of many essential oils (7). The most commonly used method (8,9) for the determination of the total ester contents is based on the separation of the acids from the saponified balsams and titration with alkali. Another method (8) for the determination of total ester in Peru balsam is based on the ether extraction of the balsam in the presence of an alkali and then drying the ether soluble matter to a constant weight.

The various ester components of Peru and Tolu balsams, particularly benzyl benzoate contribute to their medicinal and industrial

value. It becomes a necessity that each active ester constituent is estimated to determine the quality of the balsam.

Experimental

Apparatus and chemicals - NMR Spectrometer[1]; standard benzyl benzoate[2] ; standard benzyl cinnamate[3] ; ethyl benzoate[2] , as internal standard; Peru balsam[4] ; Tolu balsam [4] ; acetone-D6[5] and acetone [2] (Spectroscopic grade).

All chemical shifts reported are in reference to tetramethylsilane (TMS) at 0.00 ppm.

Assay procedure - Accurately, weigh the specified amount of sample (I, II or balsam) into glass-stoppered weighing bottles. Add the specified accurately weighed amount of ethyl benzoate and add 2 ml of acetone. Shake to dissolve and transfer about 0.8 ml into a precision NMR ube and obtain the spectrum. Integrate, at least three times, the peaks of interest and determine the average integral of each peak. The amount of I and/or II is then calculated in in the usual manner.

Results and Discussion

PMR spectra of I, II and a mixture of both esters in acetone-D6 show among other peaks two signlets for the benzyl methylene protons at 5.30 and 5.20 ppm respectively (Figures IA and 2A; Table I). In Peru

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1. Varian T-60A, 60MHz, Polo Alto; California, U.S.A.
2. B.D.H. Chemicals, Poole, England.
3. Fluka AG, Chemische Fabrik, Buchs, Switzerland.
4. Ayrton, Saunders & Co. Ltd., Liverpool & Dublin, G.B. and the Republic of Ireland.
5. Aldrich Chemical Company, Inc., Wisconsin, U.S.A.

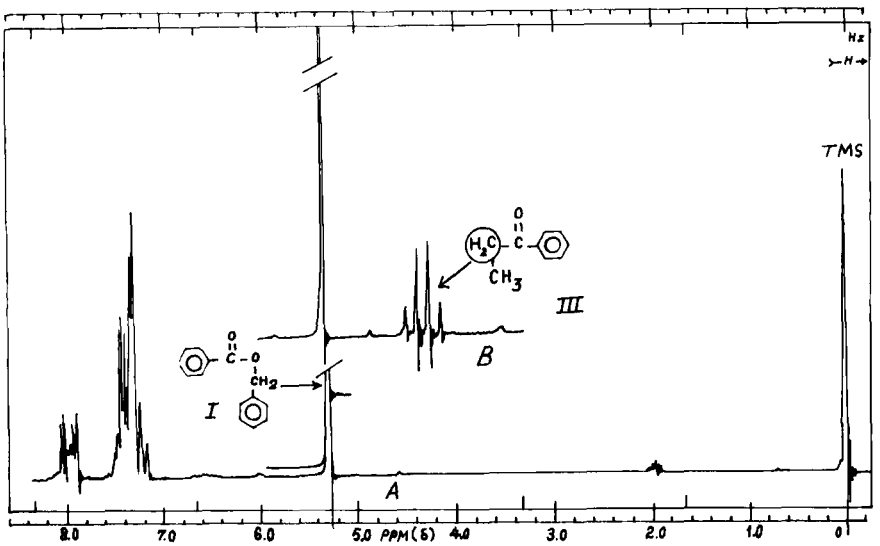


Figure 1 : A : PMR spectrum of I and TMS in acetone-D6.

B : Part of the PMR Spectrum of I, III and TMS in acetone.

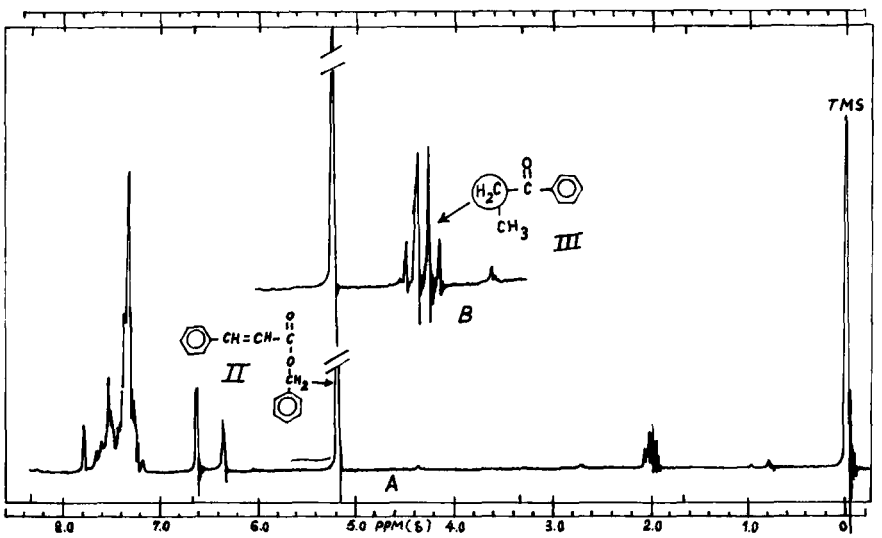
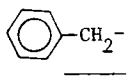


Figure 2 : A : PMR spectrum of II and TMS in acetone-D6.

B : Part of the PMR spectrum of II, III and TMS in acetone.

Table I - Chemical shift values (δ) of the esters and balsam constituents*

Functional group	Chemical shift (δ)				
	Benzyl benzoate	Benzyl cinnamate	Ethyl benzoate	Peru balsam	Tolu balsam
	7.37[a] 7.98[b]	7.33[c]	7.40[a] 7.97[b]	7.40[a] 7.93[b]	7.40[a] 7.92[b]
-CH = CH -	-	7.12[d]	-	7.23[d]	7.30[d]
	5.30[c]	5.20[c]	-	5.32[e] 5.18[f]	5.32[e] 5.18[f]
$-CH_2\underline{CH}_3$	-	-	1.37[g]	-	-
$-\underline{CH}_2CH_3$	-	-	4.32[d]	-	-
Unidentified	-	-	-	-	3.58[d] 1.13[g]

* Chemical shift values are measured in acetone-D6 with reference to TMS.

a: Eight protons multiplet, b: two protons multiplet, c: signlet,
d: AB quartet, e: singlet due to benzyl benzoate,
f: singlet due to benzyl cinnamate, g: triplet.

and Tolu balsams the corresponding peaks appear at 5.32 and 5.18 ppm. (Figures 3A and 4A). Any of the peaks assigned for the pure esters can be used for its quantitative determination (Figures 1B and 2B). However, the singlets at 5.32 and 5.18 ppm are selected for the estimation of I and II in Peru and Tolu balsams as they are free from any interference (Figures 3B and 4B). It is noteworthy that the benzyl methylene protons are not present in other balsam constituents and therefore provide a mean for specifically estimating the two esters.

Ethyl benzoate (III) is chosen as the internal standard for the assay, since it has methylene protons that provide comparable area of

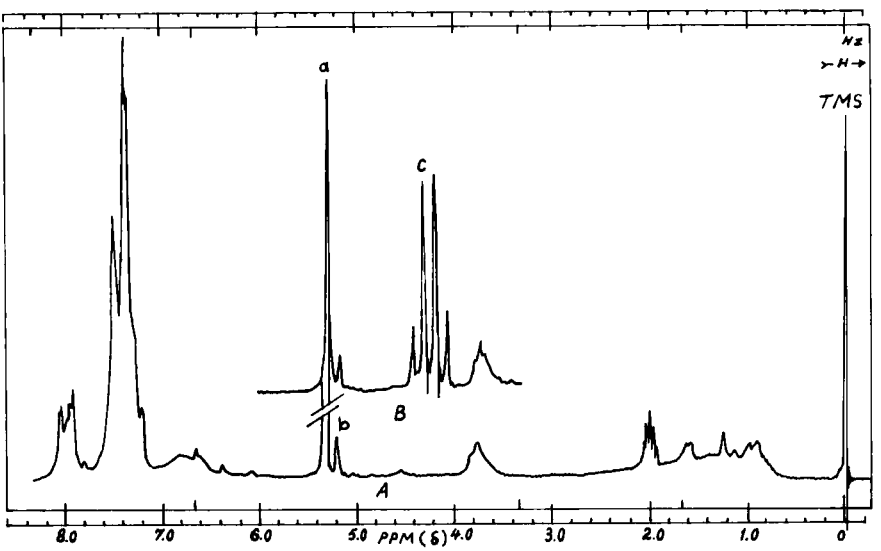


Figure 3 : A : PMR spectrum of Peru balsam and TMS in acetone-D6.

B : Part of the PMR spectrum of Peru balsam, III and TMS in acetone:

a) Singlet due to benzyl methylene of I
b) Singlet due to benzyl methylene of II
c) Quartet due to methylene of III.

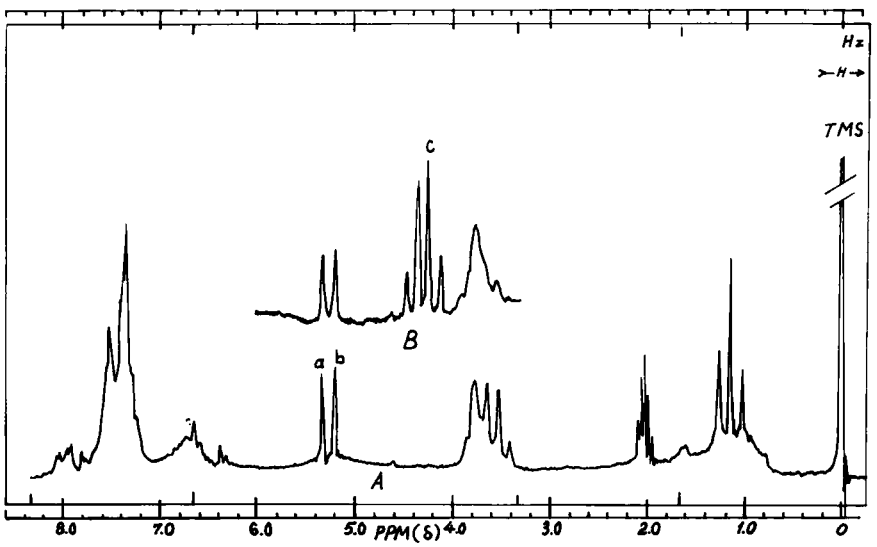


Figure 4 : A : PMR spectrum of Tolu balsam and TMS in acetone-D6.

B : Part of the PMR spectrum of Tolu balsam, III and TMS in acetone:

a) Singlet due to benzyl methylene of I
b) Singlet due to benzyl methylene of II
c) Quartet due to methylene of III.

integration to those of the benzyl methylene protons of I and II. Moreover, it displays a two methylene protons quartet centered at 4.32 ppm., which is widely separated from those of I and II (Figures 1B, 2B, Table I), thus allowing facile and accurate determination.

Acetone, rather than acetone-D6, is employed as the solvent for the assay since it is inexpensive and dissolves all balsam constituents as well as the internal standard; moreover its six protons singlet appearing at 2.07 ppm does not interfere with the lower field signals chosen for the determination of I and II.

The PMR method has been used for the determination of I and

II and the results demonstrated accurate and good precision with mean values of 100.2 ± 0.38% w/w and 100.0 ± 0.34% w/w respectively.

As the two singlets chosen for the assay of I and II are very close (Figures 3 and 4), it was necessary to assay a series of synthetic mixtures containing both pure esters to establish the accuracy of the method before applying it to the balsams (Table II). The method proved to be reproducible and accurate with mean values of 100.0 ± 0.54% w/w and 100.0 ± 0.62% w/w for I and II respectively. By applying this procedure to the commercial samples of balsams (Tables III and IV),Peru balsam was found to contain 44.7% w/w of I and 4.4% w/w of II, while Tolu balsam contained 11.6% w/w of I and 14.3% w/w of II.

Apart from its accuracy and precision the PMR method offers the advantage, over other reported procedures, of the possibility of individually quantitating the medicinally useful esters, rather than the total ester contents, and is therefore more valuable in determining the medicinal quality of the balsam.

Moreover the spectrum of the balsam provides a useful mean for estimating the exact ratio of I and II, by simply measuring their corresponding benzyl methylene protons integrals. In Peru balsam the ratio of I to II was 11.5 : 1 and in Tolu balsam it was 1 : 1.12.

The PMR spectra of the balsams are also of value to their identification, since the spectrum of Tolu balsam shows a triplet and a quartet centred at 1.13 and 3.58 ppm respectively, which are absent in Peru balsam (Figures 3A and 4A and Table I). In contrast to a previously reported finding (5), Tolu balsam was found to be devoid of ethyl benzoate as the spectrum of the latter showed a triplet centred at 1.37 ppm and quartet centred at 4.32 ppm. (Table 1). Neither this triplet nor the

Table II. Results of the determination of each component in a standard mixture containing I and II by the PMR method.

Mixture No.	Weight of internal standard* mg.	Standard Mixture of I and II					
		Benzyl benzoate (I)			Benzyl cinnamate (II)		
		Added mg.	Found mg.	% w/w	Added mg.	Found mg.	% w/w
1	23.2	55.4	55.1	99.5	74.5	24.4	99.7
2	30.9	45.2	45.0	99.6	6.1	6.1	100
3	59.0	54.4	54.9	100.8	20.8	20.7	99.5
4	59.7	81.9	81.8	99.9	8.9	9.0	101
5	67.6	97.1	96.9	99.8	9.7	9.7	100
6	55.2	50.9	51.3	100.8	144.1	142.9	99.2
7	57.8	182.2	182.1	99.9	153.8	154.7	100.6
			Average	100.0		Average	100.0
*Ethyl benzoate.			S.D.	± 0.54		S.D.	± 0.62

Table III. Results of the determination of I and II in Peru balsam by the PMR method.

Sample No.	Weight of internal standard* added. mg.	Weight of balsam mg.	Ester contents			
			Benzyl benzoate(I)		Benzyl Cinnamate (II)	
			mg.	% w/w	mg.	% w/w
1	62.3	86.0	39.1	45.5	4.0	4.6
2	74.2	141.1	63.2	44.8	6.2	4.4
3	82.1	284.3	130.3	45.8	13.0	4.6
4	83.7	187.2	81.8	43.7	7.6	4.1
5	59.4	161.5	74.6	46.0	6.9	4.3
6	79.5	202.1	89.0	44.0	9.0	4.4
7	74.6	188.0	84.1	43.1	7.9	4.2
*Ethyl benzoate.			Average	44.7	Average	4.4

Table IV. Results of the determination of I and II in Tolu balsm by the PMR method.

Sample No.	Weight of internal standard* added. mg.	Weight of Balsm mg.	Ester contents			
			Benzyl benzoate (I)		Benzyl Cinnamate (II)	
			mg.	% w/w	mg.	% w/w
1	67.0	168.1	20.4	12.1	25.5	15.2
2	74.9	215.6	26.0	12.0	29.7	13.8
3	64.7	212.2	24.5	11.5	27.6	13.0
4	69.1	207.0	22.9	11.1	28.0	13.5
5	88.4	343.5	40.2	11.7	51.8	15.1
6	64.5	241.1	29.3	12.1	37.2	15.4
7	70.0	267.2	29.3	11.0	37.2	13.9
*Ethyl benzoate			Average	11.6	Average	14.3

quartetwas shifted when ethyl benzoate was added as an internal standard for the assay of the balsams.

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