Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: www.tandfonline.com/journals/iphb20

Composition and Antifungal Activity of the Essential Oils of Caryocar brasiliensis

Xisto S. Passos, Ana Carolina M. Castro, Juliana S. Pires, Ana Cristina F. Garcia, Fernando C. Campos, Orionalda F. L. Fernandes, José R. Paula, Heleno D. Ferreira, Suzana C. Santos, Pedro H. Ferri & Maria do Rosário R. Silva

To cite this article: Xisto S. Passos, Ana Carolina M. Castro, Juliana S. Pires, Ana Cristina F. Garcia, Fernando C. Campos, Orionalda F. L. Fernandes, José R. Paula, Heleno D. Ferreira, Suzana C. Santos, Pedro H. Ferri & Maria do Rosário R. Silva (2003) Composition and Antifungal Activity of the Essential Oils of Caryocar brasiliensis, Pharmaceutical Biology, 41:5, 319-324, DOI: 10.1076/phbi.41.5.319.15936

To link to this article: https://doi.org/10.1076/phbi.41.5.319.15936

Pharmaceutical Biology
2003, Vol. 41, No. 5, pp. 319–324
1388-0209/03/4105-319$16.00

Composition and Antifungal Activity of the Essential Oils of *Caryocar brasiliensis*

Xisto S. Passos[1], Ana Carolina M. Castro[4], Juliana S. Pires[1], Ana Cristina F. Garcia[4], Fernando C. Campos[4], Orionalda F. L. Fernandes[1], José R. Paula[2], Heleno D. Ferreira[3], Suzana C. Santos[4], Pedro H. Ferri[4] and Maria do Rosário R. Silva[1]

[1]Laboratório de Micologia, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Brazil; [2]Laboratório de Farmacognosia, Faculdade de Farmácia, Universidade Federal de Goiás, Brazil; [3]Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Brazil; [4]Laboratório de Bioatividade Molecular, Instituto de Química, Universidade Federal de Goiás, Brazil

Abstract

The seed and leaf essential oils of *Caryocar brasiliensis* were analysed by GC/MS. The major constituent of the seed oil was ethyl hexanoate, while the leaf oil contained octacosane, heptadecane and hexadecanol. The pattern of geographic variation indicated that essential oils are mainly produced in lower rainfall periods and with plants grown at higher latitudes. The seed essential oil was investigated against human pathogenic systemic and opportunistic fungi using the agar dilution method. Based on the MIC values, the most significant results were obtained against *Cryptococcus neoformans* and *Paracoccidioides brasiliensis*. It was observed that the yeast-form of *P. brasiliensis* was inhibited at a concentration of 500 µg/ml, whereas 24% of *C. neoformans* strains exhibited inhibition with MIC ≤ 250 µg/ml.

Keywords: Antifungal activity, *Caryocar brasiliensis*, caryocaraceae, *Cryptococcus neoformans*, essential oil, *Paracoccidioides brasiliensis*.

List of Latin Binomials:
Candida albicans (Robin) Berkhout
Caryocar brasiliensis Camb. (Caryocaraceae)
Caryocar villosum (Aubl.) Pers.
Cryptococcus neoformans var. *neoformans* (Sanfelice) Vuillemin
Cryptococcus neoformans var. *gattii* Vanbreuseghem et Takashio
Epidermophyton floccosum (Harz) Langeron et Milochevitch
Microsporum canis Bodin
Paracoccidioides brasiliensis (Splendore) Almeida
Trychophyton mentagrophytes (Robin) Blanchard

Introduction

Paracoccidioidomycosis (PCM), caused by the dimorphic fungus *Paracoccidioides brasiliensis* (Splendore) Almeida, infects human beings through the respiratory tract in several Latin American countries, including Amerindian populations as Ecuadorian Waoroni (Davis & Yost, 1983) and Tupi-Monde from Brazilian Amazon (Coimbra Júnior et al., 1994). PCM is the main endemic systemic mycosis in Brazil (Costa et al., 1995) and can evolve to different clinical forms that are associated with various degrees of suppressed cell-mediated immunity. Additionally, the incidence of invasive diseases due to *Candida* and *Cryptococcus* species, particularly *Candida albicans* (Robin) Berkhout, *Cryptococcus neoformans* var. *neoformans* (Sanfelice) Vuillemin, and *C. neoformans* var. *gattii* VanBreuseghem et Takashio associated with acquired immune deficiency syndrome (AIDS) have increased dramatically in the last two decades in all countries (Greene, 1990). Some antifungal drugs, such as polyene macrolides and azoles, are currently used in anti-fungal therapies with certain limitations due to side effects (Kullberg, 1997). Therefore, the development of more effective and less toxic antifungal agents is required for the treatment of patients with these fungal infectious diseases (Janssen & Cauwenbergh, 1990).

Plants and their preparations have long been used among native populations of Brazilian Cerrado region as remedies against infectious diseases (Septímio, 1994). Many plant preparations have been used externally as disinfectants and antiseptics for wounds, as antidiarrhoeics and in the treatment of respiratory diseases. *Caryocar brasiliensis* Camb. (Caryocaraceae) is widely distributed in the central and southeastern regions of Brazil (Araujo, 1995) and is called

Accepted: October 16, 2002

Address correspondence to: Pedro H. Ferri, Laboratório de Bioatividade Molecular, Instituto de Química, Universidade Federal de Goiás, C.P. 131, 74001-970 Goiânia, GO, Brazil; Fax: +55 62 521 1167; E-mail: pedro@quimica.ufg.br

"pequi". Together with other *Caryocar* species (Dresen et al., 1989; Marx et al., 1997), *C. brasiliensis* is exploited for the oil content of the pulp mesocarp and the kernel of the seeds, which furnish edible fats of potential commercial value for cooking, substitute for butter, cosmetic industry and as a home-made soaps for washing clothes (Kawanishi et al., 1986). Traditionally, its leaf decoction is used as tonic, aphrodisiac and to treat liver diseases (Siqueira, 1982; Septímio, 1994). Its alcohol extracts showed antitumor activity against sarcoma 180 (Oliveira et al., 1970). The fruit pulp and seed oils have been used as a wound-healing anti-inflammatory and for the treatment of diseases of the respiratory tract, including cough, bronchial affections, asthma and pulmonary tuberculosis (Matos, 1997). It is also a good remedy for skin problems, as an external antiseptic for wounds and applied to swelling, bruises, and to promote cicatrization (Côrrea, 1984). Together with the related *C. villosum* (Aubl.) Pers., a tree native to the Amazon basin, the oils of the fruit pulp and seeds of *C. brasiliensis* are used for the treatment of dermatophytosis as ringworm (*Tinea capitis*) and athlete's foot, that is, *Tinea pedis* [fungal skin diseases caused by *Trychophyton mentagrophytes* (Robin) Blanchard, *Epidermophyton floccosum* (Harz) Langeron et Milochevitch, *Microsporum canis* Bodin, etc.] (Corrêa, 1984; Amorozo & Gely, 1988). Although triterpenes from leaves (Oliveira et al., 1968), and fatty acids from internal mesocarp and seeds have been reported (Handro & Barradas, 1971), the determination of the chemical composition and the antifungal activity of its essential oils, to our knowledge, has not yet been performed. In the present paper, we have analysed the composition of the hydrodistillate from leaves and seeds collected in different rainfall periods, and the antifungal activity of the seed oil against 28 strains of *C. albicans*, 23 strains of *C. neoformans* var. *neoformans*, and three *C. neoformans* var. *gattii* isolated from HIV-infected individuals with oral candidiases or cryptococcal meningitis, and one yeast-form of *P. brasiliensis*.

Materials and methods

Chemicals

Fluconazole (Pfizer, Belgium), amphotericin B (Bristol-Myers Squibb, USA) and itraconazole (Johnson & Johnson, USA) were suspended in sterile physiological Tris buffer (pH 7.4, 0.05 M) and included in assays as positive controls. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, USA).

Plant material

Mature fruits of *C. brasiliensis* were collected in the city of São Gonçalo do Abaeté (18°23′34″ S/45°49′6″ W), Minas Gerais State, Brazil (December 1997) and Goiânia city (16°34′24″ S/49°4′40″ W), Goiás State, Brazil (December 1998), representing the low (180 mm) and high (270 mm) rainfall periods, respectively. Leaves were collected in the cities of Paraúna (16°57′44″ S/50°28′53″ W; dry period, 0 mm) and Goiânia (Goiás State, Brazil), in August 1997 and December 1998, respectively. Voucher specimens have been deposited in the Herbarium of the Universidade Federal de Goiás (UFG), Goiás State, Brazil.

Extraction procedure

Air-dried leaves and fresh seeds of *C. brasiliensis* were separately hydrodistilled in a Clevenger-type apparatus for 5–6 h. The seed essential oils were separated from the aqueous solution, dried by treating with anhydrous Na_2SO_4, transferred into glass flasks filled to the top and kept at a temperature of −10 °C until used. Due to the scarce contents, the leaf oils were taken up in Et_2O (0.5 ml) and subsequently dried over anhydrous Na_2SO_4, evaporated under a partial vacuum and stored at −10 °C. Oil yields obtained were from 0.002–0.1% (w/w).

Microorganisms

P. brasiliensis (yeast phase, Pb01 strain) was isolated from a patient with pulmonary lesions. All the 28 strains of *C. albicans*, 23 strains of *C. neoformans* var. *neoformans* and three strains of *C. neoformans* var. *gattii* used in this study were isolates from human immunodeficiency virus-infected individuals with oral candidiases or patients with cryptococcal meningitis in the Hospital of Tropical Diseases, Goiás State, Brazil. *C. neoformans* varieties were determined in Canavanine-Glycine-Bromothymol Blue agar (Difco, USA) following the procedure described by Kwon Chung et al. (1982). *C. neoformans* var. *neoformans* (serotypes A and D) (Public Health Service, Maryland, USA) were kindly provided by Dr Claudete R. de Paula, Departamento de Microbiologia, Universidade de São Paulo and used as reference strains from previous knowledge of their sensitivity towards antifungal agents.

Chemical analysis

The essential oils were examined on a Shimadzu GC/MS model QP5050A apparatus using a DB-5 (J & W) or CBP-5 (Shimadzu) capillary column (30 m × 0.25 mm; coating thickness 0.25 µm composed of 5% phenylmethylpolysiloxane) which was programmed as follows: 60 °C for 2 min and then up to 240 °C at 3 °C/min, then to 270 °C at 10 °C/min, ending with a 4.5 min at 270 °C. The carrier gas was He at a flow rate of 1 mL/min, split mode, with ratio of 1 : 5, and injection volume of 0.5 µl (in CH_2Cl_2). Injection port was set at 225 °C and ion source temperature 250 °C. The ionisation voltage was 70 eV with scan mass range 40–450 *m/z*. The calculation of the retention indices was made through co-injection with C_8–C_{32} *n*-alkane series (Van Den Dool & Kratz, 1963). The compounds were identified by a computer search using NIST libraries of mass spectral data and by comparison of their retention indices (Adams, 1995). Compound concentrations were calculated from GC peak areas without applying corrections factors.

Antifungal testing

Antifungal activity was measured using a dilution in agar technique (Alves & Cury, 1992). The essential oil of seed obtained in December 1997 (100 mg) was solubilized in 1 ml of dimethyl sulfoxide (DMSO) and serially diluted two-fold in Yeast Nitrogen Base Phosphate agar (YNBP) (Merck, Germany) to obtain a concentration range of 1000–62.5 µl/ml. YNBP agar plates containing only DMSO diluted in the same way, which did not influence fungal growth, were included as controls. All *C. albicans* and *C. neoformans* strains were suspended in sterile physiological Tris buffer (pH 7.4, 0.05 M), homogenised and adjusted to an OD (530 nm) of 0.05 (equivalent to 1×10^6 UFC/ml). This suspension was used as the inoculum for the test in the agar plates. Fungal suspensions (3 µl) were inoculated using an automatic micropippete (Brand), and plates (diameter: 25 cm) were incubated at 37 °C for 48 h to *C. albicans* and 72 h to *C. neoformans* assays. In other assays, yeast-form of *P. brasiliensis* from stock culture (3 mm in diameter, grown on Sabouraud medium) were punched using a sterile cork-borer and were centrally placed onto the medium incorporated with the essential oil in the Petri plate and incubated at 37 °C for 15 days. Growth in diameter (in mm) of each fungus was measured at 48 h intervals. The minimal inhibitory concentration (MIC) was defined as the minimal concentration of the essential oil which completely inhibited the visible growth of the fungus. The sensitivity of *P. brasiliensis* and all cryptococcal strains to antifungal agents amphotericin B, fluconazole, and itraconazole, included as positive controls, was tested using the same technique. Duplicates were maintained for each concentration and fungus.

Statistical evaluation

The statistical significance of the geographic co-ordinates and essential oil components was calculated by using Student's *t*-test. $P < 0.05$ was regarded as statistically significant.

Results and discussion

The list of compounds in the essential oil samples is presented in Table 1. The main compound of the *C. brasiliensis* seed oils was ethyl hexanoate (70.9–91.0%), while the leaf oils contained octacosane (9.3–18.6%), heptadecane (14.9–16.3%), and hexadecanol (13.0–15.5%). The highest essential oil yield was obtained from the seed, and showed significant ($p < 0.05$) correlation with lower rainfall periods and with plants grown at higher latitudes. Additionally, seed oils of plants growing in different places within one geographic region were found to be similar in their chemical composition, irrespective of rain precipitation. Differences among the essential oil samples from the leaves were revealed qualitatively and quantitatively. The majority of the compounds found in hydrodistillates of plant leaf collected in wet periods were linear chain aliphatic hydrocarbons whose composition and concentration differed markedly from those of leaves collected in dry periods. The pattern of even *n*-alkane distribution, mostly around C_{20}–C_{28}, indicating their probable origin in the epidermis and located in the cuticular waxes (Carriere et al., 1990). It has been shown the deterrent effects of epicuticular waxes from *C. brasiliensis* on leaf-cutting ant (Sugayama & Salatino, 1995), and its important role in the resistance of some plants, since waxes constitute the first line of resistance of a plant against insect and microorganism attack (Schoonhoven et al., 1998). Among the terpenes, oxygenated monoterpenes were detected in higher concentrations than the hydrocarbons ones in all leaf populations analysed. Nevertheless, important differences between sesquiterpenes were found, which were abundant in the leaf oil from the dry season ($p < 0.05$) and absent in the wet period.

The essential oil from seeds at concentrations up to 1000 µg/ml did not show antifungal activity against any of the *C. albicans* strains tested. In contrast, the oil was active against the yeast-form cells of *P. brasiliensis* (MIC value of 500 µg/ml) and 67% of the *C. neoformans* strains at concentrations ≤1000 µg/ml (Table 2). Additionally, 24% of *C. neoformans* isolates were inhibited by the essential oil at concentrations ≤250 µg/ml. The sensitivity of the same fungal isolates to commonly used antifungal agents as amphotericin B and itraconazole afforded, respectively, MIC values at 5 and 1.25 µg/ml for *P. brasiliensis*. Additionally, 5.4% of the cryptococcal strains were resistant to amphotericin B, with 12.5 µg/ml needed for inhibition. Likewise, fluconazole and itraconazole showed MIC values at 25 µg/ml and ≥12.5 µg/ml for 8.1 and 10.8%, respectively (data not shown). These MIC values were similar to that of essential oil against 9.5% of strains isolated. Other than these, the essential oil was less potent than antifungal agents against the fungal strains tested.

The results suggest that the study of the essential oils and epicuticular waxes annual variations pattern will provide more evidence both for the biosynthetic interrelations of its constituents and for the better commercial exploitation of *Caryocar* seed oil. Its oil is ester-rich, suitable for the high-value end uses such as soap manufacture, perfumery and in aromatherapy. Additionally, the results from the present study suggest that preparations from *C. brasiliensis* could be applied in the case of fungal-induced respiratory lesions.

Acknowledgements

The authors are indebted to CNPq/PCOP (#520769/99-6), CNPq/PADCT III (#620166/97-5) and FUNAPE/UFG for

Table 1. Percentage composition of the seed and leaf oils of the *C. brasiliensis* in different rainfall periods.

		Seed		Leaf	
Compounds	KI[a]	Lower rainfall[b]	Higher rainfall[c]	Higher rainfall[c]	Dry period[d]
Monoterpene hydrocarbons		–	**0.9**	–	–
(Z)-β-Ocimene	1043	–	0.9	–	–
Oxygenated monoterpenes		–	–	**12.2**	**14.1**
Linalool	1092	–	–	3.7	7.3
α-Terpineol	1184	–	–	4.9	4.4
Nerol	1226	–	–	0.3	0.1
Geraniol	1253	–	–	3.5	2.3
Sesquiterpene hydrocarbons		–	–	–	**1.1**
(E)-Caryophyllene	1414	–	–	–	1.1
Oxygenated sesquiterpenes		–	–	–	**14.8**
(E)-Nerolidol	1561	–	–	–	1.5
Spathulenol	1572	–	–	–	0.8
Caryophyllene oxide	1578	–	–	–	7.2
Viridiflorol	1587	–	–	–	2.3
α-Muurolol	1642	–	–	–	2.0
α-Cadinol	1650	–	–	–	1.0
C_{13}-*Nor*isoprenoids		–	–	**9.2**	**12.2**
Geranyl acetone	1452	–	–	5.5	5.8
(E)-β-Ionone	1484	–	–	3.7	6.4
Fatty acid derivatives		**100.0**	**99.1**	**66.5**	**44.3**
Methyl hexanoate	918	–	1.1	–	–
Hexanoic acid	984	0.8	4.7	–	–
Ethyl hexanoate	995	91.0	70.9	–	–
Ethyl 2-hexenoate	1038	3.5	7.0	–	–
Propyl hexanoate	1091	–	0.7	–	–
Octanoic acid	1180	–	0.9	–	–
Ethyl 4-octenoate	1186	–	0.6	–	–
Ethyl octanoate	1194	3.5	7.2	–	–
Ethyl 2-octenoate	1243	1.2	4.4	–	–
Ethyl decanoate	1392	–	0.6	–	–
Ethyl 2,4-decadienoate	1465	–	0.5	–	–
Ethyl dodecanoate	1592	–	0.5	–	–
Tetradecanal	1614	–	–	1.9	–
Heptadecane	1705	–	–	16.3	14.9
Hexadecanol	1881	–	–	13.0	15.5
Ambrettolide	1925	–	–	4.3	4.6
Heneicosane	2103	–	–	8.7	–
Hexacosane	1604	–	–	3.7	–
Octacosane	2802	–	–	18.6	9.3
Total identified (%)		**100.0**	**100.0**	**87.9**	**86.5**

[a] Kovat's Index on DB-5 (seed oil) or CBP-5 (leaf oil) column.
[b] December 1997 (180 mm of water). Oil yield: 0.1%.
[c] December 1999 (270 mm of water). Oil yield: 0.004% (seed); 0.002% (leaf).
[d] August 1997 (0 mm of water). Oil yield: 0.03%.
– = not detected.

Table 2. Anti-fungal activity of essential oil from *C. brasiliensis* seeds against different *C. neoformans* isolates and reference strains.

	Cryptococcus neoformans			
	Variety		Reference strain[a]	
MIC (µg/ml)	*neoformans*	*gattii*	Serotype A	Serotype D
>1000	7 (33.3)[b]	3 (100)	1	
1000	7 (33.3)			1
500	2 (9.5)			
250	2 (9.5)			
125	1 (4.9)			
62.5	2 (9.5)			
Total	21 (100)	3 (100)	1	1

[a] *C. neoformans* var. *neoformans*.
[b] No. of strains (%).

financial support. Thanks are also due to CNPq/PIBIC-UFG for fellowships to A.C.M.C., J.S.P., A.C.F.G. and F.C.C.

References

Adams RP (1995): *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Illinois, Allured.

Alves SH, Cury AE (1992): Sensibilidade de leveduras do gênero *Candida* isoladas de pacientes com câncer, à antifúngicos poliênicos. *Rev Ins Med Trop S Paulo 34*: 251–254.

Amorozo MCM, Gely A (1988): Uso de plantas medicinais por caboclos do Baixo Amazonas, Barbarena-PA, Brasil. *Bol Mus Paraense Emilio Goeldi, Série Botânica 4*: 47–131.

Araujo FD (1995): A review of *Caryocar brasiliensis* (Caryocaraceae) – An economically valuable species of the Central Brazilian Cerrados, *Econ Bot 49*: 40–48.

Carriere F, Chagvardieff P, Gil G, Pean M, Sigoillot JC, Tapie P (1990): Paraffinic hydrocarbons in heterotrophic, photomixotrophic and photoautotrophic cell-suspensions of *Euphorbia characias* L. *Plant Sci 71*: 93–98.

Coimbra Júnior CEA, Wanke B, Santos RV, Do Valle ACF, Costa RLB, Zancope Oliveira RM (1994): Paracoccidioidin and histoplasmin sensitivity in Tupi-Monde Amerindian populations from Brazilian Amazonia. *Ann Trop Med Parasitol 88*: 197–207.

Corrêa MP (1984): *Dicionário das Plantas Úteis do Brasil*. Ministério da Agricultura. Brasília, Imprensa Nacional.

Costa EO, Diniz LSM, Netto CF, Arruda C, Dgli MLZ (1995): Delayed hypersensitivity test with paracoccidioidin in captive Latin American wild mammals. *J Med Vet Mycol 33*: 39–42.

Davis EW, Yost JA (1983): The ethnomedicine of the Waorani of Amazonian Ecuador. *J Ethnopharmacol 9*: 273–297.

Dresen H, Prasad RBN, Gulz PG (1989): Composition of lipids of piqui (*Caryocar coriaceum* Wittm) seed and pulp oil. *Z Naturforsch 44C*: 739–742.

Greene SI (1990): Treatment of fungal infections in the human immunodeficiency virus-infected individual. In: Jacobs PH, Nall L, eds., *Anti-fungal Drug Therapy*. New York, Marcel Dekker.

Handro W, Barradas MM (1971): Sobre os óleos do fruto e da semente do piqui – *Caryocar brasiliensis* Camb. (Caryocaraceae). In: Ferri MG, ed., *Simpósio sobre o Cerrado, III*. São Paulo, Edgard Blücher.

Janssen PA, Cauwenbergh G (1990): Anti-fungal therapy of the future. In: Jacobs PH, Nall L, eds., *Antifungal Drug Therapy*. New York, Marcel Dekker.

Kawanishi K, Raffauf RF, Schultes RE (1986): The Caryocaraceae as a source of fish poisons in the Northwest Amazon. *Bot Mus Leaflets, Harvard Univ 30*: 247–253.

Kullberg BJ (1997): Trends in immunotherapy of fungal infections. *Eur J Clin Microbiol Inf Dis 16*: 51–55.

Kwon Chung KJ, Polacheck I, Bennett JE (1982): Improved diagnostic medium for separation of *Cryptococcus neoformans* var. *neoformans* (serotype A and serotype B) and *Cryptococcus neoformans* var. *gattii* (serotype B and serotype C). *J Clin Microbiol 15*: 535–537.

Marx F, Andrade EHA, Maia JG (1997): Chemical composition of the fruit pulp of *Caryocar villosum*. *Z Lebensm Unters Forsch A 204*: 442–444.

Matos FJA (1997): *O Formulário Fitoterápico do Professor Dias da Rocha*. Fortaleza, Ed. Fac-Sim/EUFC.

Oliveira MM, Gilbert B, Mors WB (1968): Triterpenes in *Caryocar brasiliensis. An Acad Bras Ciênc 40*: 451–452.

Oliveira MM, Sampaio RP, Giorgi W, Gilbert B, Mors WB (1970): *Caryocar brasiliensis* – Isolamento e identificação de algumas substâncias com atividade biológica sobre o sarcoma 180. *Arq Inst Biol 37*: 25–27.

Schoonhoven LM, Jeremy T, Van Loon JJA (1998): *Insect-Plant Biology from Physiology to Evolution*. London, Chapman & Hall.

Septímio LR (1994): *A Fitoterapia Baseada em Ervas Medicinais do Cerrado*. SIPE, Brasília, Ministério da Cultura.

Siqueira JC (1982): Plantas do Cerrado na medicina popular. *J Bras Ciênc (Spectrum) 2*: 41–44.

Sugayama RL, Salatino A (1995): Influence of leaf epicuticular waxes from Cerrado species on substrate selection by *Atta sexden* rubropilosa. *Entomol Exp Appl 74*: 63–69.

Van Den Dool H, Kratz PDJA (1963): Generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromatogr 11*: 463–471.