Phytochemistry, 1980, Vol. 19, pp. 2013–2016. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0901-2013 $02.00/0

ALKALOIDS OF *UNCARIA ATTENUATA* FROM THAILAND*

DHAVADEE PONGLUX,† TANOMJIT SUPAVITA,† ROBERT VERPOORTE‡ and J. DAVID PHILLIPSON§

† Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 5, Thailand; ‡ Department of Pharmacognosy, Gorlaeus Laboratories, University of Leiden, Wassenaarseweg 76, Leiden, The Netherlands; § Department of Pharmacognosy, The School of Pharmacy, 29–39 Brunswick Square, London, WC1N 1AX, U.K.

(*Received* 26 *November* 1979)

Key Word Index— *Uncaria attenuata*; Rubiaceae; heteroyohimbine alkaloids; tetrahydroalstonine; rauniticine; 14-β-hydroxy-3-*iso*-rauniticine.

Abstract—The major alkaloids of a sample of leaves of *Uncaria attenuata* obtained from Thailand have been identified as the pentacyclic heteroyohimbine alkaloids tetrahydroalstonine, rauniticine and the novel 14-β-hydroxy-3-*iso*-rauniticine. Evidence for the structure of the new alkaloid was obtained from a study of UV, IR, MS, ^{1}H NMR and ^{13}C NMR spectra.

INTRODUCTION

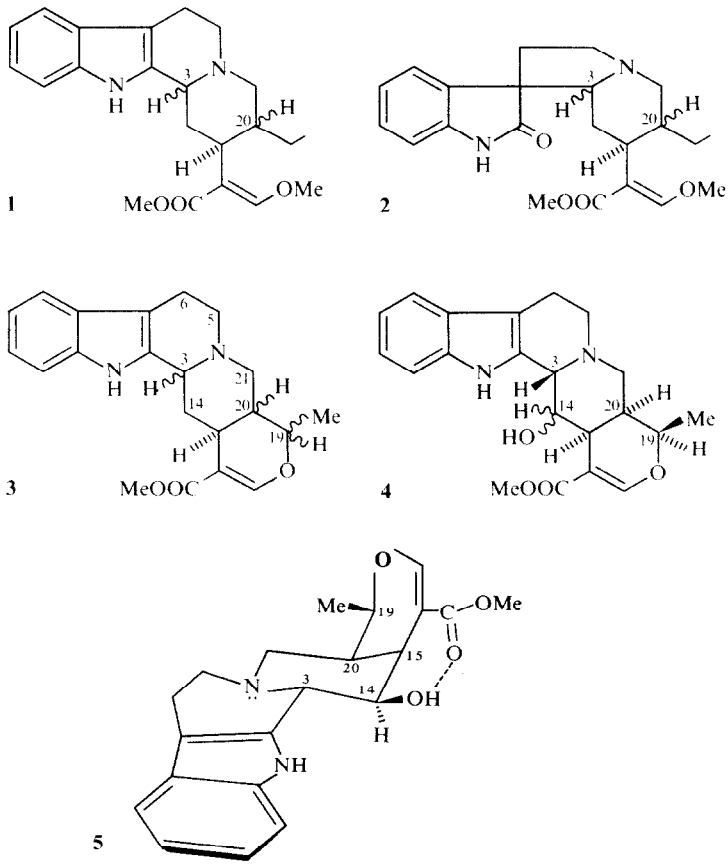
In a revision of the genus *Uncaria* [1], *U. attenuata* Korth. was not further subdivided, although previously the same author had recognized two subspecies, '*attenuata*' and '*bulusanensis*' (Elm.) Rids. msc. [2, 3]. The major alkaloids of '*U. attenuata* ssp. *attenuata*' have been reported as being either of the tetracyclic heteroyohimbine- (**1**) or the corresponding oxindole- (**2**) types with *normal* (C-3 Hα, C-20 Hβ) or *pseudo* (C-3 Hβ, C-20 Hβ) configurations [3, 4]. Pentacyclic heteroyohimbines (**3**) and their corresponding oxindole derivatives are present as minor alkaloids together with harman-, yohimbine-, yohimbine oxindole- and *pseudo* indoxyl-types [3, 4]. The alkaloids from plants previously designated as ssp. '*bulusanensis*' appear to be more variable since a tetracyclic heteroyohimbine with *epiallo* configuration (**1**, C-3 Hβ, C-20 Hα) was found as the major alkaloid in one specimen and a pentacyclic heteroyohimbine with *pseudo* configuration (**3**, C-3 Hβ, C-20 Hβ) was the major alkaloid from another [3, 4]. Many species of *Uncaria* contain identical alkaloids when different samples, often collected from widely separated geographical localities, have been examined [4] but *U. attenuata* is one of the species which is variable in its alkaloidal content. The results of alkaloid testing on 15 samples of *U. attenuata* from Sumatra, Java, Sarawak, Borneo, Sabah, Sulawesi and the Philippines, have been reported previously [3, 4], but at that time no specimens were available from Thailand. This communication describes the isolation and characterization of alkaloids from the leaves of a specimen of *U. attenuata* collected in southern Thailand.

*Part 6 in the series "Alkaloids from *Uncaria* Species". For Part 5 see Phillipson, J. D., Hemingway, S. R. and Ridsdale, C. E. (1978) *Lloydia (J. Nat. Prod.)* **41**, 503.

RESULTS AND DISCUSSION

The major alkaloids of the leaves of a Thai sample of *U. attenuata* were of the pentacyclic heteroyohimbine-type (**3**). The known alkaloids tetrahydroalstonine (**3**, *allo*, C-3 Hα, C-20 Hα, C-18 Meα) and its C-18 Meβ configuration isomer, rauniticine were identified by UV and ^{1}H NMR spectra, MS, TLC and by conversion to their C-3 Hβ analogues, akuammigine and 3-*iso*-rauniticine, respectively. The other major alkaloid was characterized as the novel 14β-hydroxy-3-*iso*-rauniticine. Hence, this particular sample of *U. attenuata* differs from the majority of other samples investigated [4] in that pentacyclic heteroyohimbines (**3**) predominated rather than the tetracyclic-types (**1**). Rauniticine has not previously been reported from the genus *Uncaria* and this is the first time that tetrahydroalstonine has been found in *U. attenuata*.

The structure of the novel alkaloid is based on the interpretation of spectral data. The UV spectrum is due to the summation of indole and Me ester vinyl ether chromophores and is indicative of a heteroyohimbine alkaloid. The MS gave a M^+ at *m/e* 368 and accurate mass measurements showed that the molecular formula was $C_{21}H_{24}N_2O_4$. Fragment ions at *m/e* 143, 144, 156, 169, 170 and 184 indicated an *ar*-unsubstituted tetrahydro-β-carboline moiety. The ^{1}H NMR spectrum showed four aromatic protons, an NH, one OMe, an olefinic proton and a three-proton doublet which could be attributed to a MeCH-moiety. Hence, the ^{1}H NMR spectrum was consistent with a pentacyclic heteroyohimbine alkaloid but such a compound would have an expected MW of 352 and not 368 as indicated by MS. A major fragment ion appeared at *m/e* 350 and accurate mass measurements corresponded to $C_{21}H_{22}N_2O_3^+$; the loss of 18 amu from the M^+ suggests the presence of a secondary OH group. A broad peak at 3350 cm^{-1} in the IR spectrum and the presence of a one-proton signal at δ 3.22 (J = 9.3 Hz) in the ^{1}H NMR would support the presence of a secondary OH



group; further confirmation was obtained by the formation of an acetyl derivative. It can be assumed that the new alkaloid is a pentacyclic heteroyohimbine (**3**) with an additional OH group which must be located at one of four possible centres, viz. C-5, C-6, C-14 or C-21.

From the low-field position of the signal for the NH in the ^{1}H NMR spectrum (δ 9.38) and the presence of a strong peak in the IR spectrum at 1665 cm^{-1}, it can be suggested that the carbonyl of the C-16 carboxyl group is hydrogen-bonded and that the OH substituent is at C-14. The signal for the C-18 Me in the ^{1}H NMR at δ 1.42 and a coupling constant $J_{19,20}$ = 3.5 Hz (i.e. C-19 H/C-20 H *cis*) would support a *cis* D/E junction with the C-18 Me configuration being β [5]. The negative Cotton effect at 283 nm in the CD spectrum suggests that the alkaloid possesses a C-3 Hβ configuration [6] and hence is 14-hydroxy-3-*iso*-rauniticine (**4**). The CD spectrum of the new alkaloid proved to be identical with that of 3-*iso*-rauniticine obtained by isomerization of natural rauniticine isolated from *U. attenuata*.

The presence of Bohlmann bands in the IR spectrum indicates that the lone pair of electrons on N-4 are *trans* to at least two hydrogens on adjacent carbons. For most heteroyohimbine alkaloids the presence of Bohlmann bands in the IR spectrum is indicative of a C-3 Hα configuration [6]. In the case of 14-hydroxy-3-*iso*-rauniticine, which possesses the C-3 Hβ configuration, N-4 must be inverted from the more usual conformation. The acetyl derivative, however, gave an IR spectrum which had no Bohlmann bands and in addition the ^{1}H NMR spectrum had a one-proton doublet at δ 4.39 which was attributed to the C-3 H being *cis* to the N-4 lone pair [6]. Thus, the C/D *trans* junction of the 14-hydroxy-3-*iso*-rauniticine changes to *cis* on preparation of the acetyl derivative. Such a change in conformation would help, in part, to explain the unusual TLC behaviour of the acetyl derivative which proved to have a lower R_f value than the parent compound, an indication of the availability of N-4 for binding to silica gel [7, 8]. The doublets at δ 3.92 ppm (J = 9.3 Hz) and at 3.22 in the ^{1}H NMR of 14-hydroxy-3-*iso*-rauniticine were assigned to the C-3 H and C-14 H, respectively. The coupling constant is indicative of *trans*-diaxial coupling between C-3 H and C-14 H while the slight broadening of the C-14 H signal shows that minimum coupling takes place with the C-15 H which is equatorial.

Support for these structural arguments was obtained from a study of the ^{13}C NMR spectra. The assignments for the ^{13}C NMR spectra, for each carbon atom, of 14-hydroxy-3-*iso*-rauniticine, its acetyl derivative, rauniticine and 3-*iso*-rauniticine, are given in Table 1. The chemical shifts assigned to 14-hydroxy-3-*iso*-rauniticine are 65.159 for C-3, 76.420 for C-14 and 37.712 ppm for C-15. The corresponding values for 3-*iso*-rauniticine occur at 55.089, 31.645 and 29.753 ppm, respectively (see Table 1) and indicate C-14 as the position of the OH substituent. Similarly the chemical shifts of the signals in the ^{13}C NMR which have been assigned to C-3, C-14 and C-15 for the acetyl derivative support location of the substituent at C-14. In the ^{13}C NMR spectrum of 14-hydroxy-3-*iso*-rauniticine the chemical shift for the signal attributed to C-6 appears at 21.792 ppm and is indicative of an inverted N-4 for the *epi-allo* configuration [9]. This contrasts with a

Table 1. ^{13}C NMR chemical shifts* of rauniticine (**3**, C-3 Hα, C-20 Hα, C-19 Hα), 3-*iso*-rauniticine, 14-β-hydroxy-3-*iso*-rauniticine (**5**) and its *O*-acetyl derivative

Carbon	Rauniticine	3-*Iso*-rauniticine		14-β-Hydroxy-3-*iso*-rauniticine	14-β-*O*-acetyl-3-*iso*-rauniticine
2	134.373	(—)	[134.5]	134.349	(—)
3	57.952	55.089	[55.0]	65.159	56.909
5	52.928	53.438	[53.4]	52.928	51.509
6	21.064	21.598	[21.7]	21.792	16.866†
7	107.118†	(—)	[105.9]	107.798†	(107.4)
8	127.116	(—)	[127.1]	126.413	(127.2)
9	117.917	117.991	[117.9]	117.699	118.031
10	119.326	119.350	[119.2]	118.744	119.400
11	121.291	121.388	[121.3]	121.073	121.764
12	110.710	110.807	[110.8]	110.978	110.571
13	135.871	(—)	[136.1]	135.755	(135.4)
14	32.348	31.645	[31.2]	76.420‡	68.466
15	29.607	29.753	[31.6]	37.712§	33.156‡
16	107.918†	(—)	[108.1]	105.080†	103.077
17	154.588	157.399	[157.3]	156.723	157.467
18	19.074	17.424	[17.3]	19.390	17.442†
19	76.466	(—)	[75.6]	73.993‡	68.466
20	34.412	37.033	[37.1]	35.043§	34.794‡
21	53.365	49.798	[49.8]	55.355	(40.0/44.0)
C on C-16	168.271	(—)	[167.9]	171.843	170.296
OMe	51.060	51.209	[51.1]	52.054	51.145
COMe	—	—	—	—	21.477

*Spectra determined from $CDCl_3$ solutions against TMS: (—) = not observed: (107.4) = not observed but calculated from spectrum: [134.5] = lit. values [11].

†, ‡, §Within a given column, these assignments may be interchanged.

value of 16.866 ppm for the acetyl derivative suggesting that a change of conformation of ring D takes place on acetylation. A study of Dreiding models indicates that the only possible structure which fits the criteria for an alkaloid with an *epi-allo* configuration, an 'inverted' N-4, a 14-OH substituent which is H-bonded to the ester carbonyl and with the C-3 H/C-14 H *trans*-diaxial, must possess a 14-β-hydroxyl configuration. Therefore the novel alkaloid is 14-β-hydroxy-3-*iso*-rauniticine (**5**).

This is the first report of a heteroyohimbine alkaloid with a OH substituent at C-14 and it is likely that other heteroyohimbines with this type of substituent will be located as natural products. Changes in configuration at C-3 of heteroyohimbine alkaloids are readily obtained by imine intermediates and it is thought that this process is utilized by plants. However, it is tempting to speculate that a further pathway may exist via dehydration of C-14 OH derivatives. Such a mechanism apparently exists for biotransformations involving changes of configuration at C-20 in which cathenamine is involved [10].

EXPERIMENTAL

U. attenuata Korth. was collected in Chumporn Province, S. Thailand, during August–September 1975. The plant material was identified by Dr. C. E. Ridsdale, Rijks-Herbarium, Leiden, and a voucher specimen is retained at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Isolation of alkaloids. Dried powdered leaves (5.1 kg) were moistened with 10% NH_4OH and macerated with EtOH (14 l.). Second and third macerations (12, 10 l.) were carried out and the combined EtOH extracts concd under red. pres. to yield a semi-solid. The residue was treated with HOAc and warm H_2O to give a final conc of 5% HOAc. The acid extract was allowed to stand overnight, filtered and the filtrate made alkaline with NH_4OH and repeatedly extracted with $CHCl_3$. The combined $CHCl_3$ extracts were concd under red. pres. and extracted with 5% H_2SO_4 (×3), made alkaline with NH_4OH and extracted into $CHCl_3$. The $CHCl_3$ extract was washed, dried and concd under red. pres. to yield a total of 45.9 g of crude alkaloid (0.9%). An aliquot (13.2 g) was dissolved in a min vol. of $CHCl_3$, mixed with Al_2O_3, air-dried and added to an Al_2O_3 column (Merck, neutral; 2.5 × 25 cm). Elution was commenced with Et_2O and continued with $CHCl_3$ and like fractions (each 10 ml) were combined on the basis of TLC comparisons as follows: (a) Et_2O, 100 ml, negative; (b) Et_2O, 20 ml, yielded tetrahydroalstonine (40 mg); (c) Et_2O, 100 ml, yielded crystals from dry Et_2O of rauniticine (0.65 g); (d) $CHCl_3$, 250 ml (5.6 g). An aliquot of fraction (d) (1 g) was added to a Si gel column (Merck; 2.5 × 25 cm) and eluted with cyclohexane-EtOAc (1:1) to yield 14-β-hydroxy-3-*iso*-rauniticine (0.22 g).

Identification of alkaloids. Rauniticine (**3**, C-3 Hα, C-20 Hα, C-19 Hα) UV λ_{max}^{EtOH} nm: 223, 284, 292. MS 70 eV *m/e* (rel. int.): 352 [M^+] (100), 351 (70), 337 (27), 323 (4), 321 (5), 293 (9), 265 (3), 251 (10), 225 (4), 223 (14), 221 (16), 209 (7), 197 (9), 184 (8), 170 (16), 169 (26), 156 (45), 143 (12), 130 (8), ratio rel. int. *m/e* 184 < 169, 170 indicative of a *cis* D/E junction [8]. ^1H NMR (60 MHz, $CDCl_3$): δ 1.47 (3 H, *d*, $J_{18,19}$ = 6.5 Hz, C-18), 3.78 (3 H, *s*, OMe), 4.45 (1 H, *m*, $J_{18,19}$ = 6.5 Hz, $J_{19,20}$ = 2.5 Hz, C-19), 7.2 (3 H, *m*, C-10, C-11, C-12), 7.47 (1 H, *d*, J_0 = 10 Hz, C-9), 7.57 (1 H, *s*, C-17). 7.95 (1 H, *br s*, NH). Identical R_f values in systems A–D (Table 2) were obtained with an authentic sample.

Table 2. R_f values of isolated and prepared alkaloids

	TLC systems*			
	A	B	C	D
Tetrahydroalstonine	82	91	85	93
Akuammigine	63	64	31	43
Rauniticine	42	43	33	43
3-*Iso*-rauniticine	68	75	55	—
14-β-Hydroxy-3-*iso*-rauniticine	70	88	70	77

* Si gel G: A, $CHCl_3$–Me_2CO (5:4); B, $CHCl_3$–EtOH (19:1); C, cyclohexane–EtOAc (1:1); D, Et_2O.

3-Iso-*rauniticine* (**3**, C-3 Hβ, C-20 Hα; C-19 Hα). Rauniticine (500 mg) dissolved in HOAc (20 ml) was heated with $Hg(OAc)_2$ (1.8 g) at 60° for 36 hr. Excess Hg^{2+} was removed from the filtered reaction mixture by precipitation with H_2S and the filtrate reacted with Zn dust (4 g) after addition of H_2O (5 ml). The reaction mixture was stirred for 48 hr at room temp., filtered, made alkaline with NH_4OH and extracted with $CHCl_3$ (3 × 20 ml). The combined $CHCl_3$ extracts were washed with H_2O, dried (Na_2SO_4), filtered and concd to dryness under red. pres. to yield an amorphous solid (510 mg), PLC of an aliquot (100 mg) with Si gel, $CHCl_3$–EtOH (1:1) yielded 3-*iso*-rauniticine (45 mg). UV λ_{max}^{EtOH} nm: 223, 284, 292. MS, 70 eV, *m/e* (rel. int.): 352 [M^+] (100), 351 (50), 337 (67), 323 (9), 321 (10), 293 (24), 251 (30), 225 (4), 223 (30), 221 (27), 209 (14), 197 (16), 184 (11), 169 (21), 156 (33), 144 (10), 143 (11). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.38 (3 H, *d*, $J_{18,19}$ = 6.5 Hz, C-18), 3.78 (3 H, *s*, OMe), 4.15 (1 H, *dd*, $J_{19,20}$ = 2.5 Hz, $J_{18,19}$ = 6.5 Hz, C-19), 7.67 (1 H, *s*, C-17), 8.62 (1 H, *br s*, NH) [11]. R_f values in systems A–D (Table 2) were identical with those of the authentic alkaloid.

Tetrahydroalstonine (**3**, C-3 Hα, C-20 Hα, C-19 Hβ). R_f values in TLC systems A–D (Table 2) were identical with those of the authentic alkaloid. UV and MS (M^+, *m/e* 352) were identical with lit. values [8].

Akuammigine (**3**, C-3 Hβ, C-20 Hα, C-19 β). Tetrahydroalstonine (44 mg) isomerized (as described above for 3-*iso*-rauniticine) to yield akuammigine (8 mg), had an identical R_f values in systems A–D (Table 2) with authentic alkaloid. UV and MS were identical with lit. values [8].

14-β-*Hydroxy*-3-iso-*rauniticine* (**5**). UV λ_{max}^{EtOH} nm: 226, 284, 291; no change on addition of NaOH. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (*s*, NH), 3350 (*br*, OH), 2850, 2810, 2750 (Bohlmann bands), 1665 (H-bonded ester CO). MS (probe) 70 eV *m/e* (rel. int.): 368 [M^+] (100), 367 (37), 353 [$M^+ - 15$] (13), 351 [$M^+ - 17$] (21), 350 [$M^+ - 18$] (57), 337 (9), 335 (10), 321 (11), 309 (19), 267 (21), 237 (13), 225 (36), 223 (30), 209 (21), 199 (21), 184 (24), 171 (56), 169 (63), 156 (40), 144 (30), 143 (26), 55 (40); m* 368 → 350; Accurate mass measured 368.1726; $C_{21}H_{24}N_2O_4$ calc. for 368.1716; measured 350.1630; $C_{21}H_{22}N_2O_3$ calc. for 350.1630. ^{1}H NMR (100 MHz, $CDCl_3$): δ 1.42 (3 H, *d*, $J_{18,19}$ = 6.8 Hz, C-18), 3.22 (1 H, *d*, $J_{3,14}$ = 9.3 Hz, C-14), 3.80 (3 H, *s*, OMe), 3.92 (1 H, *d*, $J_{3,14}$ = 9.3 Hz, C-3), 4.45 (1 H, *m*, $J_{18,19}$ = 6.8 Hz, $J_{19,20}$ = 3.5 Hz, C-19), 7.1–7.7 (4 H, *m*, C-9, C-10, C-11, C-12), 7.70 (1 H, *s*, C-17), 9.38 (1 H, *br s*, NH, exchanges with D_2O). CD (MeOH, *c* 0.2) $\Delta\varepsilon_{292}$ − 10.04, $\Delta\varepsilon_{283}$ − 12.82, $\Delta\varepsilon_{278}$ − 11.15, $\Delta\varepsilon_{256}$ + 16.17, $\Delta\varepsilon_{234}$ − 41.26, $\Delta\varepsilon_{220}$ + 47.95, $\Delta\varepsilon_{192}$ − 30.11.

Acetyl derivative. 14-β-Hydroxy-3-*iso*-rauniticine (15 mg) was dissolved in pyridine (1 ml) and Ac_2O (0.5 ml) added. After standing overnight at room temp. the solvent was evapd under red. pres. and the two major components separated by PLC (system A) to yield acetyl derivative (7.7 mg), R_f 0.24 and starting material (4 mg), R_f 0.6. IR ν_{max}^{KBr} cm^{-1}: 1730 (CO), no Bohlmann bands. ^{1}H NMR (100 MHz, $CDCl_3$): δ 1.38 (3 H, *d*, $J_{18,19}$ = 6.6 Hz, C-18), 2.19 (3 H, *s*, COMe), 3.61 (3 H, *s*, OMe), 4.09 (1 H, *q*, $J_{18,19}$ = 6.6 Hz, $J_{19,20}$ = 2.5 Hz, C-19), 4.39 (1 H, *d*, $J_{3,14}$ = 2.5 Hz, C-3), 7.0–7.4 (4 H, *m*, C-9, C-10, C-11, C-12), 7.26 (1 H, *s*, C-17), 8.09 (1 H, *br s*, NH).

Acknowledgements—We are grateful to the following for generously supplying authentic alkaloids: Dr. M. Pais, Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France (rauniticine, 3-*iso*-rauniticine); Dr. P. J. Houghton, Chelsea College, University of London (akuammigine); Dr. L. Merlini, Politecnico di Milano, Italy (tetrahydroalstonine). We thank Mr. D. Carter and Mr. W. Baldeo, respectively, for determination of MS NMR spectra.

REFERENCES

1. Ridsdale, C. E. (1978) *Blumea* **24**, 43.
2. Ridsdale, C. E. (1972) Ph.D. Thesis, University of Bristol.
3. Phillipson, J. D. and Hemingway, S. R. (1975) *Phytochemistry* **14**, 1855.
4. Phillipson, J. D., Hemingway, S. R. and Ridsdale, C. E. (1978) *Lloydia* (*J. Nat. Prod.*) **41**, 503.
5. Shamma, M. and Moss, J. B. (1962) *J. Am. Chem. Soc.* **84**, 1739.
6. Trager, W. F., Lee, C. M. and Beckett, A. H. (1967) *Tetrahedron* **23**, 365.
7. Phillipson, J. D. and Shellard, E. J. (1967) *J. Chromatogr.* **31**, 427.
8. Phillipson, J. D. and Hemingway, S. R. (1975) *J. Chromatogr.* **105**, 163.
9. Wenkert, E., Chang, C.-J., Chawla, H. P. S., Cochran, D. W., Hagaman, E. W., King, J. C. and Orito, K. (1976) *J. Am. Chem. Soc.* **98**, 3645.
10. Husson, H.-P., Kan-Fan, C., Sévenet, Th. and Vidal, J. P. (1977) *Tetrahedron Letters* 1889.
11. Melchio, J., Bouquet, A., Pais, M. and Goutarel, R. (1977) *Tetrahedron Letters* 315.