Synthesis of Various New Nitroxide Free Radical Fatty Acids†

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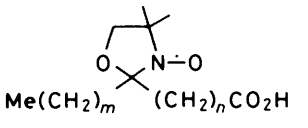
A generally applicable method is presented for the synthesis of various new nitroxide fatty acid isomers in which the fatty acid chains are attached at different positions of the pyrrolidin-1-oxyl ring. These isomers can be obtained by Michael addition of a nitroalkane to an α,β-unsaturated ketone to give a γ-nitro ketone, followed by ring closure with zinc and ammonium chloride to give a 1-pyrroline *N*-oxide which then reacts with Grignard reagents to give a pyrrolidin-1-oxyl free radical compound, which undergoes phase transfer oxidation of its terminal unsaturated bond.

Spin labelling is a powerful method for the investigation of biological membranes, which play an important role in various physiological processes.[1-3] In the course of spin labelling of membranes, the labelled fatty acids or phospholipids are incorporated into the lipid bilayers. From the anisotropy of the e.s.r. spectra of the spin labelled system, conclusions can be drawn about the structure of biomembranes, molecular motions, microenvironmental conformation relationships, and lipid–protein interactions.



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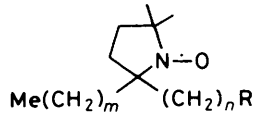

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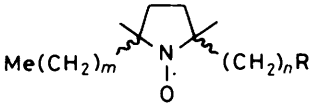

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Three types of nitroxide fatty acids (**1**),[4] (**2**),[5] and (**3**)[6] are known at present. Oxazolidine ring-containing fatty acids (**1**) are used most frequently for biological purposes because the synthesis of this type is relatively easy.[3,4] Its other advantage is that the nitroxide principal axes (*A* and *g* tensors) are parallel to the long axis of the molecule.[5] However, this type of fatty acid (**1**) also has some drawbacks: the oxazolidine ring easily suffers ring cleavage and the *N*-oxyl part of the molecule is also quite sensitive to reduction.[5]

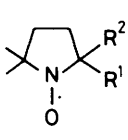
The pyrrolidine ring-containing nitroxides (**2**) and (**3**) (R = CO_2H), however, are more resistant toward reduction but their synthesis is more tiresome owing to the elaborate multistep formation of the carboxylic groups.[5-7] One of the most common methods[7] for the introduction of the carboxylic group is shown in equation (**1**).

$$R = OTHP \rightarrow OH \rightarrow O\text{-}SO_2Me \rightarrow I \rightarrow CN \rightarrow CO_2H \quad (1)$$

The 2,5-substituted pyrrolidin-1-oxyl fatty acid (**3**) has a further benefit. Its structure is closer to that of the zig-zag chains of naturally occurring saturated fatty acids, and so it causes relatively less steric perturbation during the spin labelling.[8]

† *Preliminary report:* K. Hideg and L. Lex, *J. Chem. Soc., Chem. Commun.*, 1984, 1263

The objectives of the work presented here are as follows. First of all, to report the synthesis of the new nitroxide fatty acids (**4**) and (**5**), in which two or three atoms of the pyrrolidine ring can be viewed as an integral part of a fatty acid chain. These newer isomeric nitroxide fatty acids (**4**) and (**5**), used for labelling, may also cause only minimal steric perturbance. Their long molecular axes do not correspond to any of the principal axes of the nitroxide groups, so it is possible to clarify the effect of the off-axis orientation of the fatty acid isomers on e.s.r. spectral line shapes. The second objective is to introduce new synthetic routes which are less elaborate and which are generally applicable to the preparation of the pyrrolidin-1-oxyl fatty acid isomers (**2**—**5**).



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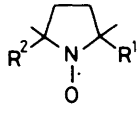

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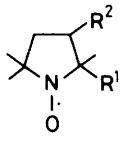

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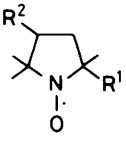
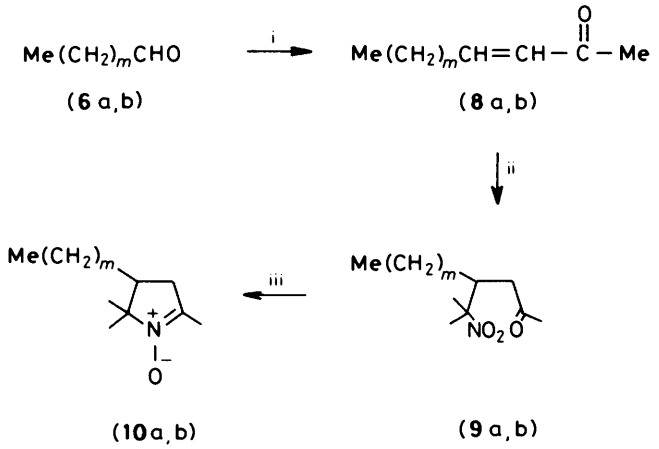


(5)

The starting compounds for the synthesis of the 2,4-substituted pyrrolidin-1-oxyl fatty acids (**5**) are the new 4-long-chain substituted 2,5,5-trimethyl nitrones (**10a,b**). The synthesis (Scheme 1) was started by the preparation of α,β-unsaturated ketones substituted at the β-position (**8a,b**) by Wittig reaction of the corresponding aldehydes (**6a,b**) and 1-triphenylphosphoranylidenepropane-2-one (**7**)[9] (Method A). The Michael addition of 2-nitropropane to (**8a,b**) was carried out in the presence of a basic catalyst [*e.g.* benzyltrimethylammonium hydroxide (Triton B)] to give the γ-nitroketones (**9a,b**) (Method B).[10] The conversion of (**9a,b**) into the corresponding nitrones (**10a,b**) was performed by a known route[11] using zinc powder and ammonium chloride (Method C).

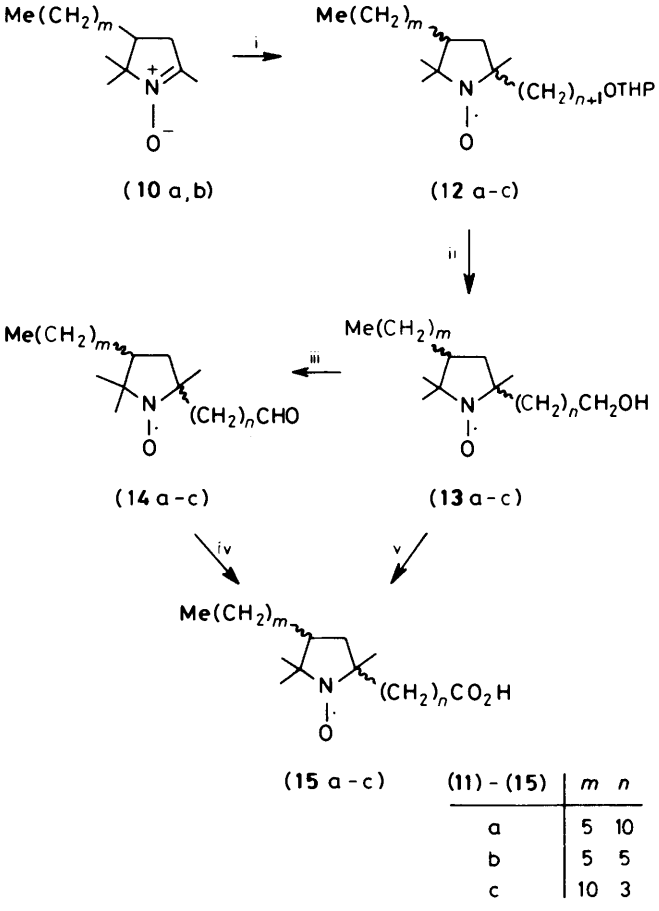
For the preparation of type (**5**) fatty acids (**15a**—**c**) (Scheme 2), the nitrones (**10a,b**) were treated with Grignard reagents (**11a**—**c**), prepared from α-hydroxy-ω-halogenoalkanes protected at their hydroxy groups with tetrahydropyranyl (THP) groups (X-[CH_2]$_n$-CH_2-OTHP), to give *N*-hydroxy derivatives which were oxidized by oxygen in the presence of Cu^{2+} ions to give the protected nitroxide compounds (**12a**—**c**) (Method D). The THP-protecting group was eliminated by treatement with toluene-*p*-sulphonic acid in methanol (Method E) to give the hydroxy derivatives (**13a**—**c**) which were oxidized by dimethyl sulphoxide activated with oxalyl chloride[12,13] to give the nitroxide aldehydes (**14a**—**c**) (Method F).

The carboxylic acids could be obtained either by the



(6), (8) - (10)	m
a	5
b	10

Scheme 1. *Reagents:* i. $MeCOCH{=}PPh_3$ (7) (Method A); ii, Me_2CHNO_2. Triton B (Method B); iii, Zn, NH_4Cl (Method C).



(11) - (15)	m	n
a	5	10
b	5	5
c	10	3

Scheme 2. *Reagents:* i. $X(CH_2)_nCH_2OTHP$ (**11a**—**c**). Cu^{2+}, O_2 (Method D): ii. TosOH (Method E): iii. Me_2SO. $(COCl)_2$ (Method F): iv. Ag_2O (Method G): v. $KMnO_4$. 18-crown-6 (Method H).

oxidation of the aldehydes (**14a**—**c**) with silver(I) oxide[14] (Method G) or directly by the oxidation of the hydroxy compounds (**13a**—**c**) with potassium permanganate in a phase transfer reaction using 18-crown-6 as catalyst (Method H).[15] In the preparation of compounds (**12**)—(**15**) the *cis*/*trans* isomers were separated only in quantities sufficient for determining the analytical data. On the basis of the literature data[6] it was expected that the Grignard reagents would prefer to attack the nitrones (**10a**,**b**) from the less hindered side, and therefore the major isomers were assigned the *trans*-geometry and the minor isomers the *cis*-geometry (see experimental section).

In the new synthetic route applied to the synthesis of the nitroxide fatty acid isomers (**24**)—(**28**) (Scheme 3) we used three modifications of the methods previously described[5,6] for the synthesis of 2,2- and 2,5-substituted nitroxides [types (**2**) and (**3**)]. First, we prepared nitrones substituted at the 3-position for the synthesis of 2,4-substituted derivatives [type (**5**)] (as in the first part of this paper); second, we synthesized nitrones substituted at the 3,4-position for use in the synthesis of 2,3-substituted nitroxides [type (**4**)]; and third, we used Grignard reagents with an unsaturated bond at the ω-position for the preparation of nitroxides from which the carboxylic group could be obtained by oxidation of the terminal unsaturated bond in the last synthetic step.

For the preparation of the different types of nitroxide fatty acids it was necessary to synthesize nitrones and nitroxides having a double bond at the end of the side chains. One of the key intermediates of this synthesis is the ω-halogenoalk-1-ene (**16**). However, the methods[16,17] previously available for preparation of this type of compound are rather elaborate ones. We therefore sought a more generally applicable and convenient method, utilizing recent procedures.

For the synthesis of the short chain ω-halogenoalk-1-enes (**16**, $n = 2$—4) a very recently described method[18] proved most suitable. According to this procedure, hexamethylphosphoramide at high temperature (195—220 °C) causes the elimination of hydrogen bromide from α,ω-dibromoalkanes to give the desired ω-halogenoalk-1-enes (**16**) (Method I) [equation (2)]. We used this method for the preparation of the derivative (**16**; $n = 4$).

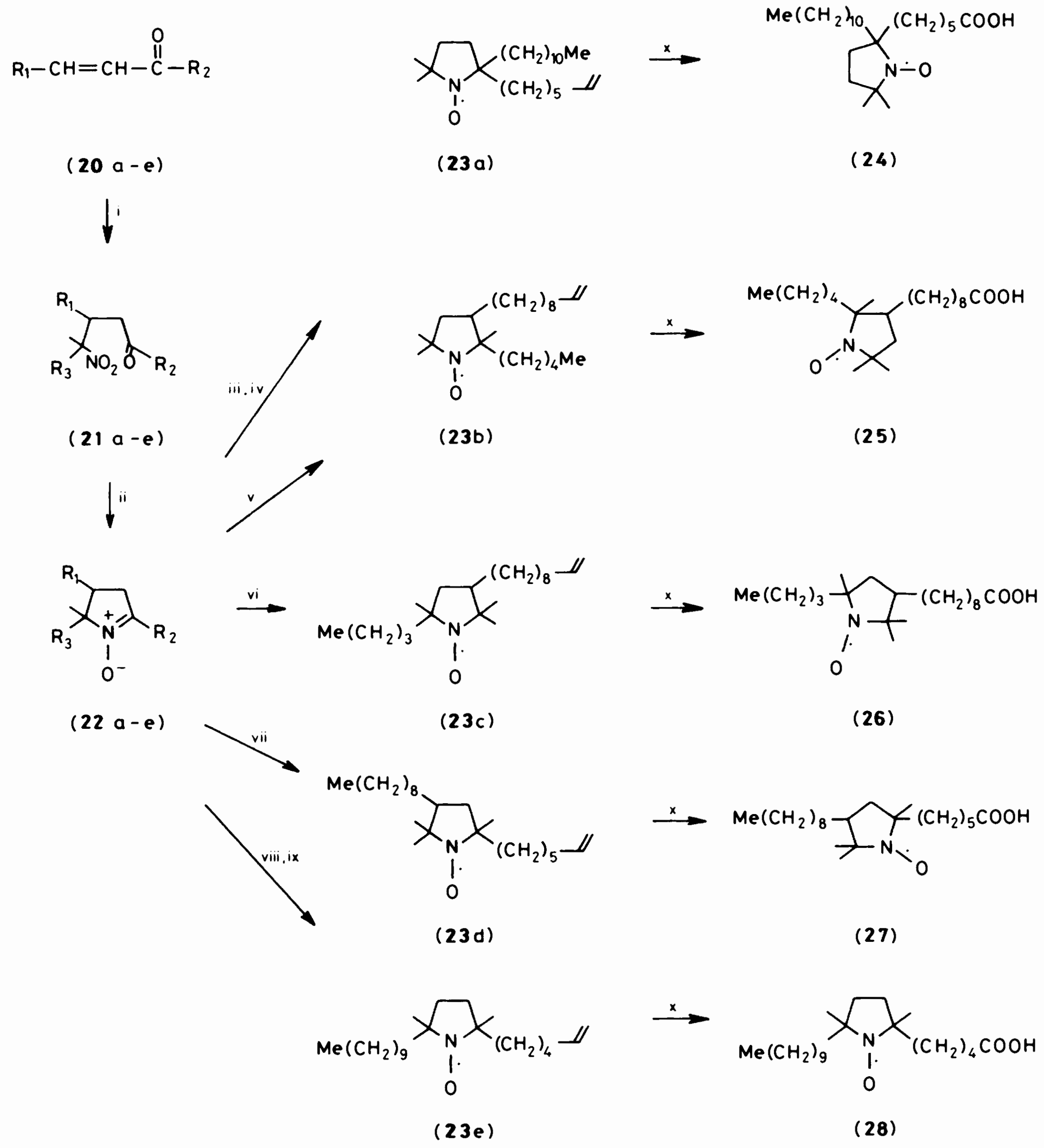
$$Br(CH_2)_nCH_2CH_2Br \longrightarrow \underset{(16)}{Br(CH_2)_nCH{=}CH_2} \quad (2)$$

The other method used for the preparation of longer chain compounds (**16**; $n = 5,9$) was based on the halogeno-polycarbon homologation reaction,[19] in which the α,ω-dibromoalkanes could be transformed with alkyl Grignard reagents to ω-bromoalkanes in the presence of dilithium tetrachlorocuprate[20] catalyst. This reaction was utilized, with allylmagnesium bromide as the Grignard reagent, to obtain the desired ω-bromoalk-1-enes (**16**) (Method J) [equation (3)].

$$CH_2{=}CHCH_2MgBr + Br(CH_2)_{n-1}Br \longrightarrow (16) \quad (3)$$

However, when the compound (**16**; $n = 5$) was prepared by this method, the product was contaminated by the starting 1,4-dibromobutane which could not be removed by fractionation. Efficient purification was achieved by chemical means, first by dibromination of the unsaturated halogen compound (**16**; $n = 5$) followed by fractional distillation of the tribromo compound (**17**; $n = 5$) and its dehydrohalogenation with zinc powder[21] to obtain the pure ω-bromoalk-1-ene (**16**; $n = 5$) [equation (4)].

$$(16) \longrightarrow \underset{(17)}{Br(CH_2)_nCHBrCH_2Br} \longrightarrow (16) \quad (4)$$



(20) - (22)	R^1	R^2	R^3
a	H	H	Me
b	$CH_2{=}CH{-}(CH_2)_8$ —	Me	$Me(CH_2)_4$ —
c	$CH_2{=}CH{-}(CH_2)_8$ —	Me	Me
d	$Me(CH_2)_8$ —	Me	Me
e	H	Me	H

Scheme 3. *Reagents:* i, $R^3CH(Me)NO_2$, Triton B (Method B); ii, Zn, NH_4Cl (Method C); iii, $Me(CH_2)_{10}MgBr$, Cu^{2+}, O_2 (Method M); iv, $CH_2{=}CH(CH_2)_5MgBr$, Cu^{2+}, O_2 (Method M); v, MeMgI, Cu^{2+}, O_2 (Method M); vi, $Me(CH_2)_3MgBr$, Cu^{2+}, O_2 (Method M), vii, $CH_2{=}CH(CH_2)_5MgBr$, Cu^{2+}, O_2 (Method M); viii, $Me(CH_2)_9MgBr$, Cu^{2+}, O_2; ix, $CH_2{=}CH(CH_2)_4MgBr$, Cu^{2+}, O_2 (Method M); x, $KMnO_4$, 18-crown-6 (Method N).

Table 1. Analytical data for α,β-unsaturated ketones, γ-nitroketones, and nitrones

Comp.	Method	Yield (%)	B.p./mmHg (°C)	R_F	Molecular formula	Found (%) (Required) C	H	N	$\nu_{max.}$ (cm^{-1})
(8a)	A	78	104—109/20[a]	0.39[b]	$C_{10}H_{18}O$	77.90 (77.87)	11.76 (11.76)		1 695, 1 675 (CO)
(8b)	A	69	106—108/0.2	0.38[b]	$C_{15}H_{28}O$	80.35 (80.29)	12.44 (12.58)		1 700, 1 680 (CO) 1 630 (C=C)
(20b,c)	A	82	94—98/0.6	0.35[b]	$C_{14}H_{28}O$	80.66 (80.71)	11.51 (11.61)		1 695, 1 670 (CO) 1 635, 1 625 (C=C)
(20d)	A	85	75—78/0.1	0.35[b]	$C_{13}H_{24}O$	79.78 (79.53)	12.37 (12.32)		1 700, 1 675 (CO) 1 625 (C=C)
(9a)	B	62	96—108/0.2	0.39[c]	$C_{13}H_{25}NO_3$	64.01 (64.17)	10.15 (10.36)	6.01 (5.76)	1 720 (CO)
(9b)	B	55	142—143/0.05	0.35[c]	$C_{18}H_{35}NO_3$	69.25 (68.97)	10.76 (11.25)	4.58 (4.47)	1 720 (CO)
(21b)	B	65	Oil	0.42[c]	$C_{21}H_{39}NO_3$	71.34 (71.34)	11.13 (11.12)	4.08 (3.96)	1 720 (CO) 1 640 (C=C)
(21c)	B	71	149—150/0.2	0.32[c]	$C_{17}H_{30}NO_3$	68.70 (68.88)	10.50 (10.20)	4.59 (4.73)	1 720 (CO) 1 640 (C=C)
(21d)	B	62	138—139/0.07	0.37[c]	$C_{16}H_{31}NO_3$	66.98 (67.33)	10.81 (10.95)	5.07 (4.91)	1 720 (CO)
(10a)	C	65	Oil	0.51[d]	$C_{13}H_{25}NO$	74.07 (73.88)	11.09 (11.92)	6.50 (6.63)	1 605 (N=C)
(10b)	C	68	Oil	0.47[d]	$C_{18}H_{35}NO$	76.72 (76.81)	12.58 (12.53)	4.70 (4.98)	1 600 (N=C)
(22b)	C	62	Oil	0.47[d]	$C_{21}H_{39}NO$	78.21 (78.44)	12.41 (12.23)	3.98 (4.36)	1 640 (C=C) 1 600 (N=C)
(22c)	C	60	Oil	0.47[d]	$C_{17}H_{31}NO$	76.90 (76.95)	11.40 (11.77)	5.09 (5.28)	1 640 (C=C) 1 595 (N=C)
(22d)	C	69	Oil	0.49[d]	$C_{16}H_{31}NO$	76.03 (75.83)	12.38 (12.33)	5.33 (5.53)	1 600 (N=C)

[a] Lit.,[22] 101—102/11 mmHg. [b] Eluant: hexane–acetone (20:1). [c] Eluant: hexane–acetone (10:1). [d] Eluant: chloroform–methanol (10:1).

The other synthesized ω-bromoalk-1-ene (**16**; $n = 9$) was purified from the 1,8-dibromo-octane contaminant by chromatography.

The ω-bromoalk-1-enes (**16**) are also key intermediates in the preparation of unsaturated aldehydes [(**19**; $n = 9$) for (**20b**)] needed for the synthesis of β-alkylated α,β-unsaturated ketones. In our hands the most convenient route for the conversion of the ω-halogenoalk-1-enes (**16**) into the ω-alkenals (**19**) was a modified Kornblum oxidation method: either by conversion of the bromide compound (**16**) with sodium iodide into a more reactive iodide derivative (**18**), then oxidization of this with dimethyl sulphoxide to give the corresponding ω-alkenal (**19**) [equation (5)] (Method K),[23] or by the direct oxidation of the bromo compound with dimethyl sulphoxide, catalysed by silver tetrafluoroborate (Method L).[24]

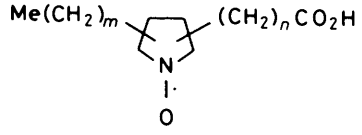
$$\textbf{(16)} \longrightarrow \underset{\textbf{(18)}}{CH_2{=}CH(CH_2)_nI} \longrightarrow \underset{\textbf{(19)}}{CH_2{=}CH(CH_2)_{n-1}CHO} \quad (5)$$

These intermediates [(**16**) and (**19**)] were used for the synthesis of various types of nitroxide fatty acids [types (**2**)—(**5**)].

Further β-alkylated, α,β-unsaturated ketones (**20b—d**) were also prepared by the Method A. The γ-nitro ketones (**21a—e**) could also be obtained by Method B. Reduction of the γ-nitro ketones (**21a—e**) gave the nitrones (**22a—e**) (Method C).

The nitrones (**22a—e**) were treated with Grignard reagents to give *N*-hydroxy derivatives, which were oxidized to the nitroxide compounds (**23a—e**) which have a terminal unsaturated bond (Method M). Phase transfer oxidation of the terminal unsaturated bond by potassium permanganate[15] led to the formation of various nitroxide fatty acids (**24—28**) (Method N).

Using the above generally applicable synthetic routes, any structural isomer of the stable nitroxide free radical fatty acid (when of course, 2,5-carbons have no H substituent) can be prepared.



Experimental

M.p.s were determined on a Boetius micro-melting point apparatus. I.r. spectra were obtained neat or as Nujol mulls on a Carl-Zeiss Specord 75 spectrometer. The e.s.r. spectra were obtained from 10^{-4}M solutions (chloroform, methanol) using a Carl-Zeiss ER-9 spectrometer. All of the monoradicals exhibit three equidistant lines with a_N = 14.3—14.8 *G*. The mass spectra were recorded with a Varian-Mat-SM-1 instrument. After work-up of the reaction mixtures the organic extracts were dried on sodium sulphate or magnesium sulphate. The purity of the new compounds was tested by t.l.c. Light petroleum refers to the fraction b.p. 40—70 °C. The starting compounds (**7**),[9] (**22a**),[25] and (**22e**)[26] were prepared by known methods and compounds (**6a,b**) were purchased from the Aldrich Chemical Company. The physical data of the new derivatives are summarized in Tables 1—3.

General Procedure for the Preparation of the α,β-Unsaturated Ketones (**8a,b**) *and* (**20b—d**). *Method A.*—A suspension of the corresponding aldehyde (**6a,b**) (**19**; $n = 9$) (0.1 mol) and 1-

Table 2. Analytical data for compounds (**12**—**15**)[a]

Compd.	Yield (%)	Method	R_F	Molecular formula	Found (%) (Required) C	H	N	$\nu_{max.}$ (cm^{-1})	*m/z* (Rel. int.)
(**12a**)	14[b]	D	0.56[c]	$C_{29}H_{56}NO_3$	74.29 (74.62)	11.89 (12.09)	2.98 (3.01)		
(**12b**) (*trans*)	15[b]	D	0.48[c]	$C_{24}H_{46}NO_3$	72.66 (72.68)	11.32 (11.69)	3.74 (3.53)		
(**12b**) (*cis*)	3[b]	D	0.42[c]	$C_{24}H_{46}NO_3$	72.50 (72.68)	11.80 (11.69)	3.68 (3.53)		
(**12c**)	17[b]	D	0.40[c]	$C_{27}H_{52}NO_3$	73.98 (73.92)	11.70 (11.95)	3.27 (3.19)		
(**13a**) (*trans*)	70[e]	E	0.49[d]	$C_{24}H_{48}NO_2$	75.43 (75.34)	12.80 (12.64)	3.20 (3.66)	3 430 (OH)	
(**13a**) (*cis*)		E	0.43[d]	$C_{24}H_{48}NO_2$	75.41 (75.34)	12.80 (12.64)	3.20 (3.66)	3 435 (OH)	[M^+ + 1] 383 (5.5), [M^+] 382 (21), 367 (42), 212 (100), 126 (26), 97 (60)
(**13b**) (*trans*)	85	E	0.50[f]	$C_{19}H_{38}NO_2$	73.02 (73.02)	13.01 (12.26)	4.29 (4.48)	3 410 (OH)	
(**13b**) (*cis*)	82	E	0.45[f]	$C_{19}H_{38}NO_2$	73.15 (73.02)	12.40 (12.26)	4.32 (4.48)	3 400 (OH)	
(**13c**) (*trans*)	71[e]	E	0.62[f]	$C_{22}H_{44}NO_2$	74.37 (74.52)	12.60 (12.51)	4.07 (3.95)	3 430 (OH)	
(**13c**) (*cis*)		E	0.43[f]	$C_{22}H_{44}NO_2$	74.45 (74.52)	12.72 (12.51)	3.96 (3.95)	3 430 (OH)	[M^+ + 1] 355 (7), [M^+] 354 (28.5), 339 (1.3), 282 (100), 266 (2.5), 210 (3.1)
(**14a**)	56	F	0.47[g]	$C_{24}H_{46}NO_2$	75.36 (75.73)	12.55 (12.18)	3.40 (3.68)	1 725 (OH)	
(**14b**) (*trans*)	52	F	0.60[g]	$C_{19}H_{36}NO_2$	73.40 (73.50)	11.47 (11.69)	4.13 (4.51)	1 730 (OH)	
(**14b**) (*cis*)	50	F	0.46[g]	$C_{19}H_{36}NO_2$	72.26 (73.50)	12.10 (11.69)	4.36 (4.51)	1 730 (OH)	
(**14c**)	61	F	0.48[g]	$C_{22}H_{42}NO_2$	74.77 (74.95)	11.83 (12.01)	3.69 (3.97)	1 725 (OH)	
(**15a**)	56	G	0.43[g]	$C_{24}H_{46}NO_3$	72.42 (72.68)	11.79 (11.69)	3.47) (3.53)	3 400—2 800 (OH) 1 700 (CO)	[M^+ + 1] 397 (2) [M^+] 396 (6.7), 381 (0.7), 212 (100), 196 (22)
(**15b**) (*trans*)	52 21	G H	0.37[g]	$C_{19}H_{36}NO_3$	69.88 (69.89)	11.04 (11.11)	3.79 (4.29)	3 400—2 700 (OH) 1 706 (CO)	[M^+ + 1] 327 (7.5), [M^+] 326 (33), 312 (5), 212 (100), 196 (10)
(**15b**) (*cis*)	37	G	0.33[g]	$C_{19}H_{36}NO_3$	69.83 (69.39)	10.80 (11.11)	4.27 (4.29)	3 400—2 700 (OH) 1 706 (CO)	[M^+] no, 296, 196
(**15c**)	57 18	G H	0.34[g]	$C_{22}H_{42}NO_3$	71.50 (71.69)	11.62 (11.49)	3.17 (3.80)	3 400—2 700 (OH) 1 705 (CO)	[M^+ + 1] 369 (15), [M^+] 368 (50), 353 (4), 282 (100), 266 (115), 210 (9)

[a] All of these compounds are oil purified by chromatography. [b] Overall yields based on γ-nitro ketones (**9a,b**). [c] Eluant: hexane–ethyl acetate (5:1). [d] Eluant: hexane–ethyl acetate (2:1). [e] Total yield of isomers. [f] Eluant: hexane–ethyl acetate (1:1). [g] Eluant: ether–light petroleum–acetic acid (4:4:0.1).

triphenylphosphoranylidenepropan-2-one (**7**) (31.8 g, 0.1 mol) in dry tetrahydrofuran (THF) (200 ml) was stirred and refluxed for 48 h. During this time the phosphorane (**7**) dissolved. The solvent was evaporated, and the residue was suspended in hot, light petroleum and then filtered. The filtrate was evaporated and the oily product was distilled.

General Procedure for the Preparation of the γ-Nitro Ketones (**9a,b**) *and* (**21b—d**). *Method B.*—To a stirred solution of the α,β-unsaturated ketones (**8a,b**), (**20b—d**) (0.05 mol) and 2-nitropropane (8.9 g, 0.1 mol) or 2-nitroheptane (14.5 g, 0.1 mol) in ether (50 ml) was added Triton B catalyst (40% solution in methanol; 4 ml) and the mixture was refluxed for 48 h. It was then washed successively with aqueous hydrochloric acid, 10% aqueous sodium carbonate, water, and saturated aqueous sodium chloride, and then dried, filtered, and evaporated. The residual oil was distilled.

General Procedure for the Preparation of Nitrones (**10a,b**) *and* (**22b—d**). *Method C.*—To a stirred suspension of the γ-nitro ketones (**9a,b**), (**21b—d**) (0.03 mol) and ammonium chloride (1.6 g, 0.03 mol) in water (40 ml) was added zinc powder (7.8 g, 0.12 mol) in small portions over a period of 2 h at such a rate that the reaction temperature did not exceed 10 °C. The reaction mixture was stirred at 0 °C for an additional 1 h, then at 20 °C for 2 h, and filtered. The filtrate was combined with several methanol washes of the filter cake. The filtrate was concentrated under reduced pressure, and then saturated with sodium sulphate and extracted with chloroform (3 × 30 ml). The chloroform extracts were dried and evaporated. The residual oil was carefully dried, by azeotropic removal of water by benzene followed by the evaporation of the remaining trace of benzene under high vacuum (*ca.* 0.05 mmHg). The nitrones were pure according to t.l.c. [chloroform–methanol (10:1); R_F = 0.47—0.51].

General Procedure for the Preparation of Tetrahydropyranyl Ethers of the Long-chain Nitroxide Alcohols (**12a—c**). *Method D.*—A solution of the nitrone (**10a,b**) (0.01 mol) in dry THF (5 ml) was added at 20 °C to a stirred solution of the Grignard

Table 3. Analytical data for compounds (**23**)—(**28**)[a]

Compd.	Method	Yield (%)	R_F	Molecular formula	Found (%) (Required) C	H	N	$\nu_{max.}$ (cm^{-1})	*m/z* (Rel. int.)
(**23a**)	M	13	0.47[b]	$C_{24}H_{46}NO$	79.00 (79.05)	12.80 (12.72)	4.00 (3.84)	1 640 (C=C)	[M^+ + 1] 365 (7.8), [M^+] 364 (25), 349 (1.5), 268 (100), 210 (100), 127 (12.5)
(**23b**)	M	19	0.39[b]	$C_{22}H_{42}NO$	78.62 (78.51)	12.54 (12.58)	4.08 (4.16)	1 640 (C=C)	[M^+ + 1] 337 (4.5), [M^+] 336 (17), 321 (2.1), 266 (100), 250 (2.8)
(**23c**)	M	14	0.41[b]	$C_{21}H_{40}NO$	78.19 (78.20)	12.27 (12.50)	3.98 (4.34)	1 640 (C=C)	
(**23d**)	M	11	0.40[b]	$C_{23}H_{44}NO$	79.01 (78.49)	12.37 (12.65)	4.01 (4.00)	1 640 (C=C)	[M^+ + 1] 351 (2), [M^+] 350 (7.6), 310 (8.8), 254 (100), 182 (3.2)
(**23e**)	M	12	0.51[b]	$C_{22}H_{44}NO$	79.18 (78.51)	12.34 (12.58)	5.07 (4.26)	1 640 (C=C)	[M^+ + 1] 337 (0.5), [M^+] 306 (13), 254 (20), 180 (20), 44 (100)
(**24**)	N	14	0.41[c]	$C_{23}H_{44}NO_3$	72.06 (72.20)	11.74 (11.59)	3.60 (3.66)	3 400—2 800 (OH) 1 700 (CO)	[M^+ + 1] 383 (9.5), [M^+] 382 (33.5), 268 (100), 228 (90), 212 (28)
(**25**)	N	15	0.43[c]	$C_{21}H_{40}NO_3$	70.97 (71.14)	11.31 (11.37)	4.07 (3.95)	3 400—2 800 (OH) 1 705 (CO)	[M^+ + 1] 355 (0.6), [M^+] 354 (2.6), 340 (1.7), 284 (100), 165 (28.5), 135 (34)
(**26**)	N	13	0.41[c]	$C_{20}H_{38}NO_3$	70.52 (70.54)	11.64 (11.75)	4.20 (4.11)	3 400—2 800 (OH) 1 700 (CO)	[M^+] 340 (5), 326 (3), 284 (100), 270 (25), 268 (26), 87 (24)
(**27**)	N	11	0.39[c]	$C_{22}H_{40}NO_3$	(71.70 (71.69)	11.60 (11.48)	4.25 (3.80)	3 400—2 800 (OH) 1 706 (CO)	[M^+ + 1] 369 (4), [M^+] 368 (12.2), 354 (6), 254 (100), 238 (10), 69 (13)
(**28**)	N	19	0.39[c]	$C_{21}H_{40}NO_3$	70.93 (71.14)	11.09 (11.37)	4.00 (3.95)	3 400—2 800 (OH) 1 700 (10)	[M^+ + 1] 355 (7), [M^+] 354 (23), 254 (96), 214 (100), 196 (38), 55 (93)

[a] All of these compounds are oils purified by chromatography. [b] Eluant: hexane–ethyl acetate (10:1). [c] Eluant: ether–light petroleum–acetic acid (4:4:0.1).

reagent [prepared from magnesium (0.61 g, 0.025 mol) and the tetrahydropyranyl ethers of α-hydroxy-ω-halogeno-alkanes* (**11a**—**c**) (0.025 mol) in dry THF (20 ml)] and stirred for 2 h. The reaction was quenched with saturated aqueous ammonium chloride, and water and ether (50 ml) were then added. The organic phase was separated, washed with saturated aqueous sodium chloride, dried, and evaporated to dryness. The residue was taken up in methanol (50 ml), 25% aqueous ammonia (0.5 ml) and a catalytic amount of copper(II) acetate were added, and the mixture was stirred for 24 h. During this time the colour of the reaction mixture changed from yellow to deep green. The reaction mixture was evaporated, and the residue was dissolved in chloroform (50 ml), washed with saturated aqueous sodium hydrogen carbonate, dried, and evaporated; the residue was then chromatographed on a silica gel column with chloroform–ether (50:1) as eluant. After two separations by chromatography it was possible to separate the *cis*/*trans* isomers of the (**12b**) derivative in pure form. The ratio of isomers was *cis*:*trans* = 1:5. Since separation of the isomers of compounds (**12a**) and (**12c**) was unsuccessful, even on t.l.c., the following reaction was carried out with the mixture of isomers.

* They were prepared as follows: to a solution of the α-hydroxy-ω-halogeno-alkane [11-bromodecan-1-ol, 6-chlorohexan-1-ol and 4-chlorobutan-1-ol (0.1 mol)] in dichloromethane (40 ml) were added 3,4-dihydro-2*H*-pyran (11.5 g, 0.13 mol) and toluene-*p*-sulphonic acid (5 mg), and the mixture was stirred at 25 °C for 3 h. The mixture was then washed with 1M aqueous sodium hydroxide, dried, evaporated and distilled under reduced pressure to give (**11a**) (50%), b.p. 148—152 °C/0.2 mmHg; (**11b**) (75%), b.p. 95—98 °C/1 mmHg; (**11c**) (80%), b.p. 62—64 °C/0.15 mmHg.

General Procedure for the Preapration of the Long-chain Nitroxide Alcohols (**13a**—**c**). *Method E.*—To a stirred solution of the nitroxide tetrahydropyranyl ether (**12a**—**c**) (0.5 mmol) in methanol (5 ml) was added a catalytic amount of toluene-*p*-sulphonic acid at 20 °C, and the mixture was stirred for 3 h before being diluted with chloroform (30 ml), washed with saturated aqueous sodium hydrogen carbonate, dried, and evaporated. For the preparation of (**13b**), pure isomers of (**12b**) were available as starting compounds so that pure *cis*- and *trans*-isomers of **13b**) were obtained. The separation of the isomers of compounds (**13a**) and (**13c**) only gave quantities sufficient for analytical analysis [chloroform–ether (4:1) and (10:1)]. Separations on a preparative scale failed, so the mixture of isomers was used in the next reaction.

General Procedure for the Preparation of the Long-chain Nitroxide Aldehydes (**14a**—**c**). *Method F.*—To a stirred solution of oxalyl chloride (25.6 μl, 0.3 mmol) in dichloromethane (1 ml) was added dropwise at −60 °C dimethyl sulphoxide (46.2 μl, 0.65 mmol), followed after 10 min at −60 °C by a similar addition of a solution of the corresponding nitroxide alcohol (**13a**−**c**) (0.25 mmol) in dichloromethane (0.5 ml). After an additional 10 min, triethylamine (181 μl, 1.3 mmol) was added and the reaction mixture was allowed to warm to room temperature. The reaction mixture was diluted with dichloromethane (15 ml) and then washed successively with 1M aqueous hydrochloric acid, water, saturated aqueous sodium hydrogen carbonate, and water, before being dried and evaporated to dryness. The residue was purified by preparative t.l.c. with chloroform–ether (7:1) as eluant. The separation of *cis*/*trans*

isomers failed even by analytical t.l.c. except in the preparation of compound (**14b**), where the starting materials were the isomers of (**13b**).

In this reaction some starting alcohols (**13a—c**) (15—20%) were always recovered.

General Procedures for the Preparation of the 2,5-*Substituted Fatty Acids* (**15a—c**).—*Method G.* To a stirred solution of the nitroxide aldehyde (**14a—c**), (0.1 mmol) in ethanol (0.5 ml) was added silver nitrate (40 mg) in water (50 μl). To the reaction mixture was added dropwise potassium hydroxide (0.5 ml, 6%). Stirring was continued for 30 min after which water was added and the mixture washed with ether (2 × 5 ml). The aqueous phase was acidified with 1M aqueous hydrochloric acid, extracted with chloroform, and the extract dried and evaporated. The residue was purified by preparative t.l.c. with ether–light petroleum–acetic acid (4:4:0.1) as eluant.

Method H. To a stirred solution of nitroxide fatty acid alcohol (**13a—c**) (0.2 mmol) in benzene (20 ml) were added potassium permanganate (0.048 g, 0.3 mmol) and 18-crown-6 (0.053 g, 0.2 mmol) and the mixture was stirred for 24 h. The reaction mixture was filtered and the black precipitate was washed with 1M aqueous sodium hydroxide. The aqueous filtrate was acidified with 1M aqueous hydrochloric acid, extracted with chloroform, and the extract dried and evaporated. The residue was purified by preparative t.l.c. with ether–light petroleum–acetic acid (4:4:0.1) as eluant.

6-*Bromohex*-1-*ene* (**16**; $n = 4$). *Method I.*—To the 1,6-dibromohexane (24.4 g, 0.1 mol), hexamethylphosphoramide (17.9 g, 0.1 mol) was added dropwise (1 drop/s) with stirring at 195 °C (in an oil-bath). The temperature was then raised to 220 °C for 5 min and the product was distilled into a receiver cooled by solid CO_2. The crude product was redistilled to give the product (**16**; $n = 4$) (8.3 g, 51%), b.p. 47—48 °C/20 mmHg (lit.,[18] b.p. 149—150 °C/760 mmHg).

7-*Bromohept*-1-*ene* (**16**; $n = 5$). *Method J.*—A solution of the Grignard reagent prepared from magnesium (12.15 g, 0.5 mol) and allyl bromide (30.21 g, 0.25 mol) in ether (200 ml) was added with stirring at 0 °C to a solution of 1,4-dibromobutane (54 g, 0.25 mol) in THF (200 ml) containing dilithium tetrachlorocuprate (2.5 mmol) prepared from lithium chloride (0.21 g, 5 mmol) and copper(II) chloride (0.34 g, 2.5 mmol). The reaction mixture was stirred for an additional 1 h after which 5% aqueous sulphuric acid was added dropwise. The organic phase was separated and washed successively with water, saturated aqueous sodium hydrogen carbonate, water, and saturated aqueous sodium chloride; the extract was then dried, evaporated, and distilled. To the distillate (b.p. 66—74 °C/ 12 mmHg, in chloroform, bromine was added with stirring at 0 °C until the colour of the mixture became orange. The reaction mixture was washed with 10% aqueous sodium hydroxide and water, and then dried, evaporated, and distilled to give the tribromo derivative (**17**; $n = 5$) (54 g, 64% based on allyl bromide), b.p. 110—114 °C/0.6 mmHg (Found: C, 24.85; H, 4.05; Br, 71.3. $C_7H_{13}Br_3$ requires C, 24.95; H, 3.89; Br, 71.16%).

The tribromo compound (**17**; $n = 5$) (54 g) was added dropwise to a stirred suspension of zinc powder (15 g) in methanol (50 ml). The reaction mixture was stirred and refluxed for 30 min and then filtered, evaporated, and distilled to give the product (**16**; $n = 5$) (24 g, 54% based on allyl bromide), b.p. 73—74 °C/19 mmHg (lit.,[16] b.p. 77—81 °C/20 mmHg) (Found: C, 47.45; H, 7.25; Br, 44.9. $C_7H_{13}Br$ requires C, 47.48; H, 7.40; Br, 45.12%).

11-*Bromoundec*-1-*ene* (**16**; n = 9). *Method J.*—A solution of the Grignard reagent [prepared from magnesium (1.21 g, 0.05 mol) and allyl bromide (3.02 g, 0.025 mol) in ether (20 ml)] was added with stirring at 0 °C to a solution of 1,8-dibromo-octane (6.8 g, 0.025 mol) in THF (20 ml) containing dilithium tetrachlorocuprate (0.1M in THF; 2.5 ml). The reaction mixture was stirred for an additional 1 h and then 5% aqueous sulphuric acid was added dropwise. The organic phase was separated, washed with water, and saturated aqueous sodium chloride, dried, and evaporated. The residue was flash chromatographed[27] on a silica gel column with hexane as eluant to give two derivatives. The first compound eluted from the column was the desired product (**16**; $n = 9$) (2.3 g, 40%) b.p. 83—84 °C/1 mmHg (lit.,[17] b.p. 103 °C/4 mmHg). The second material was the starting 1,8-dibromo-octane (2.8 g, 41%).

Undec-10-*enal* (**19**; n = 9).—*Method K.* 11-Bromoundec-1-ene (**16**; $n = 9$) (2.33 g, 0.01 mol) was added to a stirred solution of sodium iodide (3.0 g, 0.02 mol) in dry acetone (15 ml). The reaction mixture was stirred for 3 h, and then poured into water and extracted with ether. The ether phase was dried and evaporated to give the crude 11-iodoundec-1-ene (**18**; $n = 9$), which was added at 150 °C under nitrogen to a stirred mixture of dimethyl sulphoxide (40 ml) and sodium hydrogen carbonate (5 g). After 4 min the mixture was rapidly cooled and then poured into water. The aqueous solution was extracted with ether (4 × 50 ml). The combined extracts were washed with water, dried, filtered, and evaporated. The residue was flash chromatographed on silica gel column with hexane–ethyl acetate (20:1) as eluant to give the product (**19**; $n = 9$) (1.2 g, 72%), b.p. 64—65 °C/1 mmHg (lit.,[28] b.p. 101—103 °C/10 mmHg).

Method L. 11-Bromoundec-1-ene (**16**; $n = 9$) (0.47 g, 2 mmol) was added at room temperature to a stirred solution of silver tetrafluoroborate (0.426 g, 2.2 mmol) in dry dimethyl sulphoxide (2.5 ml). Stirring was continued for 48 h and then triethylamine (0.25 ml) was added. After 15 min the reaction mixture was worked up and purified as in Method K, to give the same product (**19**; $n = 9$) (0.25 g, 76%).

General Procedure for the Preparation of the Nitroxides with a Terminal Unsaturated Bond (**23a—e**).*Method M.*—The nitrone (**22a—e**) (0.02 mol) was added to a solution of the Grignard reagent {prepared from magnesium (0.58 g, 0.024 mol) and the corresponding alkyl halide [1-bromoundecane, iodomethane, 1-bromobutane, 7-bromohept-1-ene (**16**; $n = 5$), 1-bromodecane] (0.024 mol)} in dry ether (100 ml). The reaction mixture was stirred at 20 °C for 2 h, and then saturated aqueous ammonium chloride (5 ml) and water (5 ml) were added. The organic phase was separated, washed with saturated aqueous sodium chloride, dried, and evaporated. The residue was taken up in methanol (50 ml) containing 25% aqueous ammonia (3 ml) and copper(II) acetate (0.2 g) and stirred under oxygen until the reaction mixture developed a deep blue colour [in the preparation of (**23a,e**)] or a green colour [in the preparation of (**23b—d**)]. The solvent was then evaporated and the residue was taken up in chloroform, washed with saturated aqueous sodium hydrogen carbonate and water, dried, evaporated and, for the preparation of (**23b—d**), chromatographed on a silica gel column with carbon tetrachloride–ether (50:1) as eluant. In the preparation of (**23a,e**), after work-up another nitrone was obtained which was not purified but was dried by the azeotropic removal of water with benzene.

This nitrone was added to a solution of the Grignard reagent [prepared from magnesium (0.58 g, 0.024 mol), 7-bromohept-1-ene (**16**; $n = 5$), (0.02 mol) and 6-bromohex-1-lene (**16**; $n = 4$) (0.02 mol), respectively] in dry ether (100 ml). The reaction mixture was stirred for 2 h and worked up as above. After the oxidation step (in the presence of ammonia and copper(II)

acetate under oxygen) the residue oil was chromatographed on a silica gel column with carbon tetrachloride–ether (50:1) as eluant.

General Procedure for the Preparation of Various Nitroxide Fatty Acids (**24**—**28**). *Method N.*—To a stirred solution of the nitroxides (**23a**—**e**) (0.5 mmol) in benzene (10 ml) was added potassium permanganate (0.24 g, 1.5 mmol) and 18-crown-6 (0.40 g, 1.5 mmol). The reaction mixture was stirred at 20 °C for 24 h and then filtered. The unchanged starting nitroxides (**23a**—**e**) (0.1—0.3 mmol) were recovered from the filtrate. The residue was suspended, washed with 5% aqueous sodium hydroxide, and then filtered. The filtrates were acidified with hydrochloric acid, extracted with chloroform, dried, and evaporated. The residue was purified by preparative t.l.c. with ether–light petroleum–acetic acid (4:4:0:1) as eluant.

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