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Original paper

Synthesis and cardiotonic activity of esters of 2-substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acids. Crystal structure of 2-methyl,2-*t*-butyl and 2-phenyl esters*

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Summary — The synthesis of ethyl and methyl esters of 2-substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acids by reaction of ethyl or methyl 2-dimethylaminomethylene-3-oxoalkanoates with sodium cyanoacetamide is described. These esters gave by alkaline hydrolysis the corresponding carboxylic acids, which were decarboxylated to 6-substituted 1,2-dihydro-2-oxo-3-pyridinecarbonitriles.

As milrinone analogues, nearly all the above compounds were tested on contractile activity and frequency rate of spontaneously beating atria and electrically driven left atria from guinea pigs. Among the esters, ethyl 5-cyano-1,6-dihydro-2-methyl-6-oxo-3-pyridinecarboxylate induced positive inotropic and chronotropic effects superior to those caused by milrinone. By increasing or branching the 2-substitutent, the activity decreased until faded or even reversed. Carboxylic acids and nitriles were less active than milrinone.

Some aspects of the structure-activity relationship of these compounds are discussed on the basis of X-ray structural analyses of 2-methyl, 2-*t*-butyl and 2-phenyl esters.

Résumé — Synthèse et activité cardiotonique d'esters de cyano-5 dihydro-1,6 oxo-6 pyridine-3 acides carboxyliques substitués en 2. Structure cristalline des esters méthyl-2, *t*-butyl-2 et phényl-2. *On a synthétisé une série d'esters éthyliques et méthyliques de cyano-5 dihydro-1,6 oxo-6 pyridine-3 acides carboxyliques substitués en 2 par réaction des diméthylaminométhylène-2 oxo-3 alkanoates correspondants avec le cyanoacétamide sodé. Les esters ont été saponifiés et les acides obtenus décarboxylés pour donner les dihydro-1,2 oxo-2 pyridine-3 carbonitriles respectifs substitués en 2. Les résultats pharmacologiques réalisés sur presque tous ces composés apparentés au milrinone ont révélé que l'ester éthylique du cyano-5 dihydro-1,6 méthyl-2 oxo-6 pyridine-3 acide carboxylique a présenté une activité inotrope et chronotrope supérieure au milrinone. La substitution du groupe méthyl-2 avec des groupes plus lourds ou plus ramifiés entraîne une baisse ou une inversion de l'activité. La structure aux rayons X des esters méthyl-2, t-butyl-2 et phényl-2 est rapportée et pour ces composés des relations structure-activité ont été discutées.*

milrinone analogues / 2-substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acid esters / cardiotonic agents / X-ray structures

Introduction

Amrinone and milrinone are bipyridine derivatives with well established inotropic activity and vasodilatatory properties [1, 2]. They are often referred to as non glycoside, non sympathomimetic positive inotropic agents. Initially it was proposed that the mechanism of their cardiac action was an inhibition of phosphodiesterase. However other mechanisms are possible.

In the attempt to obtain new compounds with more defined mechanisms of action and consequently with more specific therapeutic indications, we have synthesized a number of analogues of milrinone, in which the 4-pyridyl moiety of the parent drug was substituted with ester and carboxylic acid groups or was even absent, whereas the methyl group was increased by omologation, ramification and aromatic substitution.

In order to get some information on structure-activity

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relationship of the above esters, the crystal structures of compounds possessing different levels of cardiotonic activity such as 2-methyl, 2-*t*-butyl and 2-phenyl esters were investigated.

Chemistry

In the field of our research on the reaction of *sym*-2-dimethylaminomethylene-1,3-diones with dinucleophiles [3], we have already described the synthesis of 5-acyl-1,2-dihydro-2-oxo-3-pyridinecarbonitriles by reaction of the above synthons with sodium cyanoacetamide.

These pyridones can be considered as analogues of milrinone where the 4-pyridyl moiety is substituted with an acyl group (for a preliminary account of the cardiotonic activity of these compounds see ref. [4]).

More recently, we extended the above reaction with dinucleophiles to unsymmetrical 2-dimethylaminomethylene-1,3-diones such as ethyl and methyl 2-dimethylaminomethylene-3-oxoalkanoates **1a–g**; in the reaction with phenylhydrazine, these synthons gave the esters of 5-substituted 1-phenyl-1H-pyrazole-4-carboxylic acids in high yields and as sole products [5]. Consequently, we have now reacted synthons **1a–g** with sodium cyanoacetamide at room temperature in dry ethanol to obtain ethyl or methyl esters of 2-substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acids **2a–g** (Table I) in satisfactory yields and as sole products. Also synthon **1e**, whose carbonyl group is strongly hindered, afforded the ester **2e** in

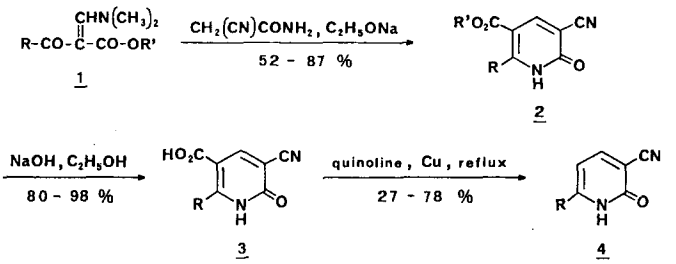
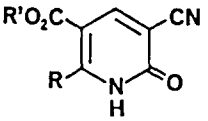


Table I. Esters of 2-substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acids **2a–g**.



Comp.	R	R'	Yield %	M.p. (a) °C	Molecular Formula	UV, λ_{max}^{nm} (log ε)	IR, cm^{-1}	^{1}H-NMR, δ
2a	CH_3	C_2H_5	67	217–218(b)	$C_{10}H_{10}N_2O_3$	260(4.20) 326.5(4.00)	3200–2400,2240, 1702,1660 (c)	1.30(t,J=7.2,ethyl CH_3), 2.61(s,CH_3-6), 4.24 (q,J=7.2,CH_2),8.37 (s,CH-4),≈13.5 (br s,NH; disappears with D_2O) (e).
2b	C_2H_5	C_2H_5	66	193–194	$C_{11}H_{12}N_2O_3$	260(4.19) 328(4.01)	3200–2600,2245 1725,1668 (d)	1.37 and 1.39 (2t,J=7.2,2 CH_3), 3.21 (q,J=7.2,$MeCH_2$-6),4.36 (q,J=7.2,$MeCH_2$O), 8.52(s,CH-4),≈12 (br s,NH; disappears with D_2O) (f).
2c	$(CH_2)_2CH_3$	C_2H_5	82	200–201	$C_{12}H_{14}N_2O_3$	260(4.19) 328(4.03)	3200–2600,2235 1720,1665 (d)	0.9–2.1 (m,2CH_3+propyl CH_2-2), 3.16 (near t, J=7.2, propyl CH_2-1),4.36(q,J=7.2,ethyl CH_2), 8.54 (s, CH-4),≈12.7 (br s, NH; disappears with D_2O) (f).
2d	$CH(CH_3)_2$	C_2H_5	87	171–172	$C_{12}H_{14}N_2O_3$	260(4.14) 327.5(4.00)	3200–2700,2235, 1720, 1663 (d)	1.2–1.6 [m, ethyl CH_3 + $(CH_3)_2C$], 4.0–4.6 (m, $CHMe_2$), 4.36 (q, J = 7.2, CH_2), 8.47 (s, CH-4),≈12.3(br s, NH; disappears with D_2O) (f).
2e	$C(CH_3)_3$	C_2H_5	70	145–147; 158–159	$C_{13}H_{16}N_2O_3$	253.5(3.88) 331.5(3.93)	3385,3200–2850, 2237,1725,1662 (d)	1.39 (t, J = 7.2, ethyl CH_3), 1.55 [s, $C(CH_3)_3$] , 4.36 (q, J = 7.2, CH_2), 8.07 (s, CH-4), 11.05 (br s, NH; disappears with D_2O) (f).
2f	C_6H_5	C_2H_5	69	254–255	$C_{15}H_{12}N_2O_3$	260.5(4.15) 336.5(4.07)	3200–2500,2240, 1700, 1650 (c)	0.93 (t, J = 7, CH_3), 3.97 (q, J = 7, CH_2), 7.50 (s, C_6H_5), 8.52 (s, CH-4), 13.17 (br s, NH; disappears with D_2O) (e).
2g	$CH_2C_6H_5$	CH_3	55	210–211	$C_{15}H_{12}N_2O_3$	262(4.13) 329.5(4.04)	3200–2500,2230 1725, 1675 (c)	3.75 (s, CH_3), 4.40 (s, CH_2), 7.30 (s, C_6H_5), 8.50 (s, CH-4), 13.27 (br s, NH; disappears with D_2O) (e).

[a]From 95% ethanol. [b]Lit. [6, 7, 24], 208°C; lit. [25], 212–213°C; lit. [26], 218°C. [c]In KBr. [d]In $CHCl_3$. [e]In DMSO–d_6. [f]In $CDCl_3$.

70% yield. To our knowledge, the sole compound of this series described in the literature was **2a**, which was obtained by Errera [6, 7] by reaction of Claisen's synthon ethyl 2-ethoxymethylene-3-oxobutanoate with sodium cyanoacetamide, and was recently prepared in low yield from the crude synthon **1a** and cyanoacetamide in the presence of sodium hydride in tetrahydrofuran [8].

Esters **2a–g** were converted to the corresponding 2-substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acids **3a–g** (Table II) by saponification with potassium hydroxide in boiling ethanol followed by acidification, generally in 80–98% yields. The 33% yield obtained in the case of **3e** is clearly attributable to the strong hindering effect of *t*-butyl group.

Finally, decarboxylation of acids **3a–g** by refluxing in quinoline containing a catalytic amount of copper powder [3] afforded 6-substituted 1,2-dihydro-2-oxo-3-pyridinecarbonitriles **4a–g** (Table III) in 27–78% yields.

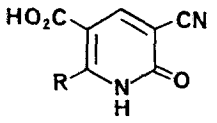
These compounds are largely known, being easily available by reaction of an α-hydroxymethyleneketone sodium salt with cyanoacetamide [9–14]; therefore, the identity of their physical data with those described in the literature, as well as the spectral data (Table III), confirmed their structure and consequently that of esters **2**.

*Crystal structure of esters **2a**, **2e** and **2f***

*Ester **2a**.* The molecular conformations of the four crystallographically independent molecules which define the unit cell of the crystal are shown in Figures 1 and 2 which provide also, due to the space group (P1) requirements, the packing arrangement.

The crystallographically independent molecules were related in pairs by pseudo inversion centers. Their geometries were very similar as was expected. The main difference among the molecules was confined to the slightly dif-

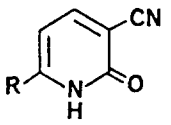
Table II. 2-Substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acids **3a–g**.



Comp.	R	Reflux time (hours)	Yield %	M.p.(a) °C	Molecular Formula	UV, λ_{max} nm ($\log \varepsilon$)	IR, cm^{-1} (KBr)	^{1}H-NMR, δ (DMSO-d_6)
3a	CH_3	4	98	302–303(dec)	$C_8H_6N_2O_3 \cdot H_2O$	257(4.14) 328(3.98)	3300–2400,2250, 1713,1658	2.62 (s, CH_3), ~6.1(br s, NH + H_2O; disappears with D_2O), 8.40 (s, CH-4), ~12.5 (br s, CO_2H; disappears with D_2O).
3b	C_2H_5	4	95	299–300(dec)	$C_9H_8N_2O_3$	256(4.10) 329.5(4.00)	3200–2300,2240, 1695, 1643	1.19 (t, J = 7.2, CH_3), 3.01 (q, J = 7.2, CH_2), 8.45 (s, CH-4), ~12.8 (br s, NH+ + CO_2H; disappears with D_2O).
3c	$(CH_2)_2CH_3$	4	80	277–278	$C_{10}H_{10}N_2O_3$	256(4.09) 330(4.01)	3300–2300,2240, 1695,1660	0.93 (t, J = 7.2, CH_3), 1.57 (m, CH_2), 2.99 (t, J = 7.2, CH_2), 8.46 (s, CH-4), ~12.9 (br s, NH + CO_2H; disappears with D_2O).
3d	$CH(CH_3)_2$	4	93	303–304(dec)	$C_{10}H_{10}N_2O_3$	254.5(4.07) 331 (3.99)	3300–2400,2235, 1692, 1657	1.26 (d, J = 7.2, 2 CH_3), 4.26 (h, J= 7.2 $CHMe_2$), 8.43 (s, CH-4), ~12.6 (br s, NH + CO_2H; disappears with D_2O).
3e	$C(CH_3)_3$	50	33	264–265	$C_{11}H_{12}N_2O_3$	241.5(3.92) 340(3.98)	3300–2400,2230, 1715,1655	1.43 [s, $(CH_3)_3C$], 8.21 (s, CH-4), ~12.5 (br s, NH + CO_2H; disappears with D_2O).
3f	C_6H_5	12	98	284–285	$C_{13}H_8N_2O_3$	257(4.09) 346.5(4.09)	3300–2300,2240, 1675,1640	7.50 (s, C_6H_5), 8.53 (s, CH-4), 13.88 (br s, NH + CO_2H; disappears with D_2O).
3g	$CH_2C_6H_5$	8	98	254–255	$C_{14}H_{10}N_2O_3$	255.5(4.07) 332.5(4.04)	3300–2400,2235, 1688, 1665	4.48 (s, CH_2), 7.33 (s, C_6H_5 + NH), 8.53 (s, CH-4), ~12.5 (br s, CO_2H; disappears with D_2O).

[a] From 95% ethanol.

Table III. 6-Substituted 1,2-dihydro-2-oxo-3-pyridinecarbonitriles **4a–g**.



Comp.	R	Reflux time (hours)	Yield %	M.p. °C	Molecular formula	UV, λ_{max} nm (log ε)	IR, cm^{-1} (KBr)	^{1}H-NMR, δ (DMSO-d_6)
4a	CH_3	3	57	303-305(dec) (a)(b)	$C_7H_6N_2O$	235 (3.84) 334 (4.03)	3200-2400, 2225,1665	2.29 (s, CH_3), 6.21 (d, J = 7.2, CH-5), 8.00 (d, J = 7.2, CH-4), ~ 12.5 (br s, NH; disappears with D_2O).
4b	C_2H_5	2	27	247-249(dec) (a)(c)	$C_8H_8N_2O$	235.5(3.85) 335 (4.07)	3200-2500, 2225,1643	1.16 (t, J = 7, CH_3), 2.56 (q, J = 7, CH_2), 6.24 (d, J = 7.8, CH-5), 8.06 (d, J = 7.8, CH-4), ~12.5 (br s, NH; disappears with D_2O).
4c	$(CH_2)_2CH_3$	3	34	151-152 (d)(e)	$C_9H_{10}N_2O$	235 (3.84) 335 (4.07)	3200-2400, 2215,1660	0.92 (t, J = 7.2, CH_3), 1.57 (m, CH_2), 2.55 (t, J = 7.2, CH_2), 6.22 (d, J = 6.6, CH-5), 8.01 (d, J = 6.6, CH-4), 12.60 (br s, NH; disappears with D_2O).
4d	$CH(CH_3)_2$	2	50	209-210 (a)(f)	$C_9H_{10}N_2O$	235(3.83) 335(4.06)	3200-2600, 2225,1660	1.22[d, J = 6.6, $(CH_3)_2C$], 2.88 (m, $CHMe_2$), 6.27 (d, J = 8.4, CH-5), 8.09(d, J = 8.4, CH-4), 12.53 (br s, NH; disappears with D_2O).
4e	$C(CH_3)_3$	3	78	204-206 (d)(g)	$C_{10}H_{12}N_2O$	232.5(3.83) 333.5(4.06)	3200-2500, 2230,1658	1.32[s, $(CH_3)_3C$], 6.30 (d, J = 7.8, CH-5), 8.06 (d, J = 7.8, CH-4), 8.85 (br s, NH; disappears with D_2O).
4f	C_6H_5	4	58	298-300(dec) (a)(h)	$C_{12}H_8N_2O$	251(3.99) 356(4.19)	3200-2400, 2230,1658	6.78 (d, J = 7.8, CH-5), 7.58 (m, 2H ar m + + 1H ar p), 7.83 (m, 2H ar o), 8.21 (d, J = 7.8, CH-4), 12.1-13.2 (br s, NH; disappears with D_2O).
4g	$CH_2C_6H_5$	3	70	196-197 (a)	$C_{13}H_{10}N_2O$	236(3.85) 336.5(4.11)	3200-2400, 2225, 1658.	3.93 (s, CH_2), 6.16 (d, J = 7.2, CH-5), 7.35 (s, C_6H_5), 7.60 (br s, NH; disappears with D_2O), 8.00 (d, J = 7.2, CH-4).

[a]From 95% ethanol. [b]Lit. [9], mp 292–294°C. [c]Lit. [10], mp 244–246°C. [d]From anhydrous diethyl ether. [e]Lit. [11], mp 153°C. [f]Lit. [12], mp 207–208°C. [g]Lit. [13], mp 203–206°C. [h]Lit. [14], mp 292–293°C.

ferent orientation of the ester groups with respect to the pyridone planes. The values of the dihedral angles between the hexa-atomic ring and the respective C-COO- moieties were 2.4(2)°, 15.6(2)°, 1.7(1)° and 13.6(2)° for molecules I, II, III and IV, respectively. The pyridone moieties were parallel to each other, the dihedral angles among their best mean planes ranged from 0.8(1)° to 2.4(1)°.

The main peculiarity of the solid state structure was the intermolecular hydrogen bond interactions between the pyridone N proton of one molecule and the ring CO oxygen of the molecule pseudo-centrosymmetrically related, with formation of octa-atomic rings in the 2 independent pairs of dimers (Fig. 1). The values for the significant interatomic distances and angles are reported in Table IV, while in Table VII some significant intermolecular distances are shown.

These values are indicative of strong interactions between molecules I and IV, and II with III. These "dimers" bear additional interactions between them. The more planar molecules (I and III) were faced at distances shorter than the normal Van der Waals distances (the contacts are on the order of 3.4 Å), thus suggesting π interactions between them.

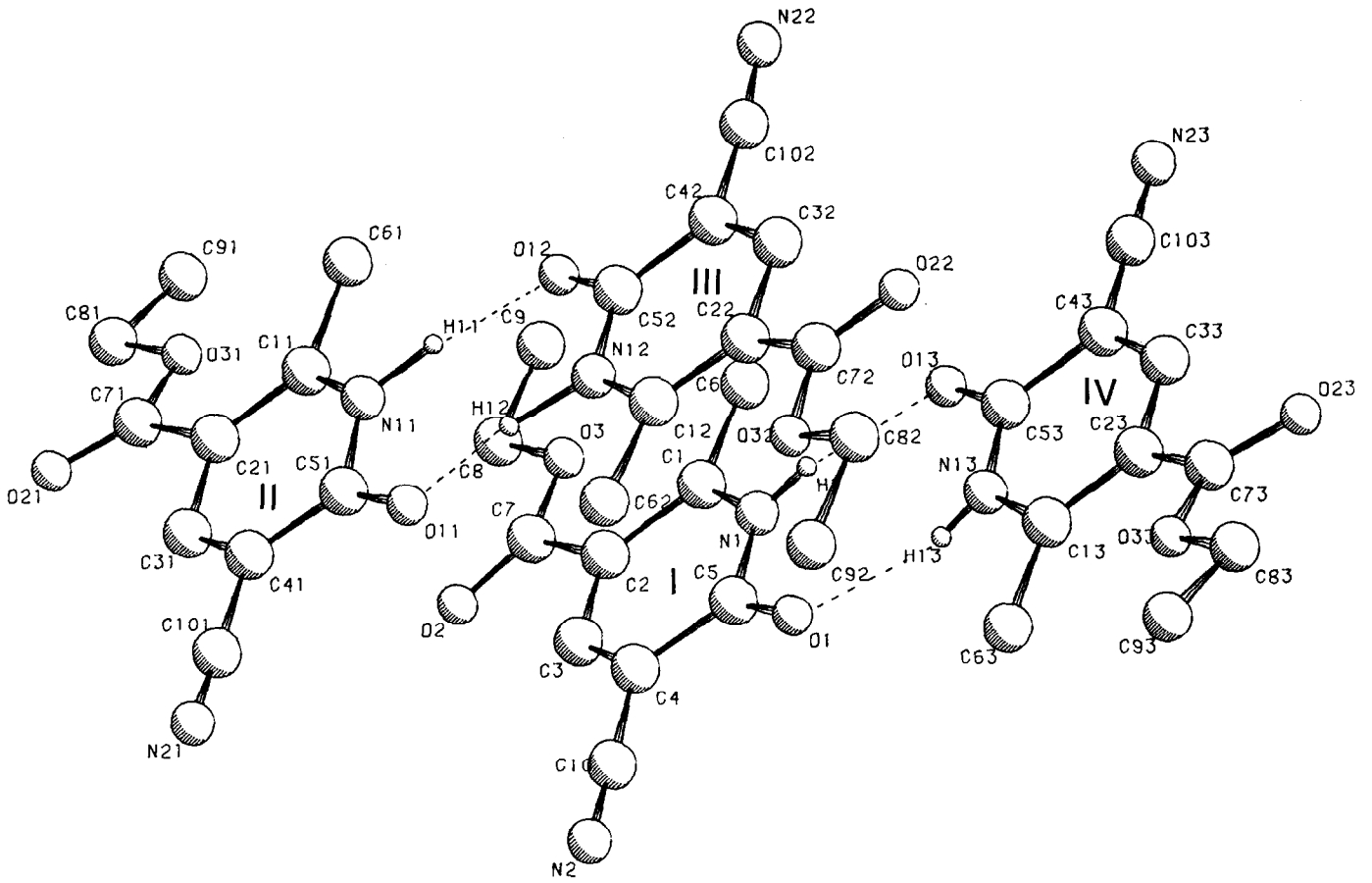


Fig. 1. Perspective view of the 4 crystallographically independent molecules of the 2-methyl derivative **(2a)** (for sake of clarity only the N–H proton is shown).

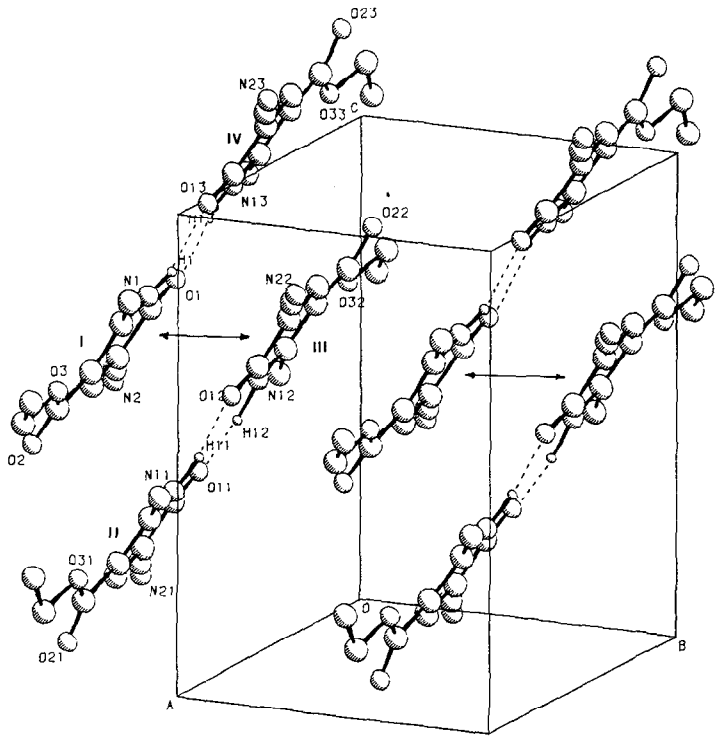


Fig. 2. Packing diagram showing the molecular stacking of the 2-methyl derivative **(2a)**.

Ester ***2e.*** This compound crystallizes in a monoclinic cell where the asymmetric unit was a dimer, formed by two molecules of the *t*-butyl derivative (compared with the ester **2a**, it differed for the *t*-butyl instead of the methyl group). Figure 3 shows a perspective view of the dimer along with the numbering scheme used for atom labelling.

Significant bond distances and angles are reported in Table V.

The dimer was formed *via* hydrogen bonds between the pyridone N proton and the ring CO oxygen of the adjacent molecule.

The conformation of each constituent of the dimer was characterized by a significant lack of planarity of the pyridone rings, in particular the N(11)...C(51) ring. The deviations from the best mean plane in the N(1)...C(5) ring ranged from −0.052(5) to 0.046(5) Å, while the second [N(11)...C(51)] ranged from −0.224(5) to 0.191(5) Å. The ester moiety was no longer "quasi" coplanar with the pyridone ring as is **2a**, but was twisted by 131.9(2)° to the ring N(1)...C(5) and the second ester moiety with respect to the N(11)...C(11) ring was twisted by 49.5(2)°.

Table IV. Selected interatomic bonds and angles for the 2-methyl derivative **(2a)**.

a) Selected bond distances (Å) for **2a**.

N(1)-H(1)	0.86(5)	N(1)-C(1)	1.352(6)
N(1)-C(5)	1.416(5)	N(2)-C(10)	1.140(6)
O(1)-C(5)	1.206(6)	O(2)-C(7)	1.179(5)
O(3)-C(7)	1.283(5)	O(3)-C(8)	1.490(8)
C(1)-C(2)	1.431(5)	C(1)-C(6)	1.477(6)
C(2)-C(3)	1.356(6)	C(2)-C(7)	1.537(8)
C(3)-C(4)	1.327(7)		
C(4)-C(5)	1.456(6)	C(4)-C(10)	1.444(6)
C(8)-C(9)	1.416(7)		
N(11)-H(11)	1.08(4)	N(11)-C(11)	1.289(6)
N(11)-C(51)	1.423(5)	N(21)-C(101)	1.128(6)
O(11)-C(51)	1.237(6)	O(21)-C(71)	1.175(5)
O(31)-C(71)	1.286(5)	O(31)-C(81)	1.466(6)
C(11)-C(21)	1.377(5)	C(11)-C(61)	1.542(5)
C(21)-C(31)	1.419(5)	C(21)-C(71)	1.548(7)
		C(31)-C(41)	1.363(7)
C(41)-C(51)	1.383(6)	C(41)-C(101)	1.470(6)
		C(81)-C(91)	1.495(8)
		N(12)-H(12)	1.16(4)
N(12)-C(52)	1.348(5)	N(12)-C(12)	1.371(6)
N(22)-C(102)	1.142(6)	O(12)-C(52)	1.268(6)
O(22)-C(72)	1.217(5)	O(32)-C(72)	1.369(5)
O(32)-C(82)	1.463(7)	C(22)-C(32)	1.457(5)
C(22)-C(12)	1.354(5)	C(22)-C(72)	1.428(7)
C(32)-C(42)	1.403(7)		
C(42)-C(52)	1.416(6)	C(42)-C(102)	1.443(5)
		C(12)-C(62)	1.517(5)
C(82)-C(92)	1.549(6		
N(13)-H(13)	0.76(4)		
N(13)-C(53)	1.349(6)	N(13)-C(13)	1.436(7)
O(13)-C(53)	1.243(7)	N(23)-C(103)	1.147(6)
O(33)-C(73)	1.381(6)	O(23)-C(73)	1.255(6)
C(13)-C(23)	1.388(5)	O(33)-C(83)	1.485(9)
C(23)-C(33)	1.392(6)	C(13)-C(63)	1.457(6)
C(33)-C(43)	1.365(7)	C(23)-C(73)	1.410(8)
C(43)-C(103)	1.411(6)	C(43)-C(53)	1.481(5)
		C(83)-C(93)	1.450(9)

b) Selected bond angles (°) for **2a**.

C(1)-N(1)-C(5)	129.5(4)	H(1)-N(1)-C(5)	111(3)
H(1)-N(1)-C(1)	119(3)	C(7)-O(3)-C(8)	117.3(4)
N(1)-C(1)-C(6)	116.2(4)	N(1)-C(1)-C(2)	114.3(4)
C(2)-C(1)-C(6)	129.4(5)	C(1)-C(2)-C(7)	122.5(4)
C(1)-C(2)-C(3)	118.8(4)	C(3)-C(2)-C(7)	118.7(4)
C(2)-C(3)-C(4)	126.0(4)		
		C(3)-C(4)-C(10)	126.0(4)
C(3)-C(4)-C(5)	119.6(4)	C(5)-C(4)-C(10)	114.4(4)
O(1)-C(5)-C(4)	127.0(5)	N(1)-C(5)-C(4)	111.8(4)
N(1)-C(5)-O(1)	121.3(4)		
		O(3)-C(7)-C(2)	115.1(4)
O(2)-C(7)-C(2)	117.7(5)	O(2)-C(7)-O(3)	126.9(5)
O(3)-C(8)-C(9)	109.6(5)		
N(2)-C(10)-C(4)	177.6(5)	C(11)-N(11)-C(51)	127.0(4)
H(11)-N(11)-C(51)	113(2)	H(11)-N(11)-C(11)	120(2)
C(71)-O(31)-C(81)	113.8(3)	N(11)-C(11)-C(61)	114.8(4)
N(11)-C(11)-C(21)	118.9(4)	C(21)-C(11)-C(61)	126.3(4)
C(11)-C(21)-C(71)	126.7(4)	C(11)-C(21)-C(31)	118.3(4)
C(31)-C(21)-C(71)	115.0(4)	C(21)-C(31)-C(41)	120.3(4)
C(31)-C(41)-C(101)	118.9(4)	C(31)-C(41)-C(51)	122.0(5)
C(51)-C(41)-C(101)	119.1(4)	O(11)-C(51)-C(41)	127.8(5)
N(11)-C(51)-C(41)	113.5(4)	N(11)-C(51)-O(11)	118.7(4)
O(31)-C(71)-C(21)	112.5(4)	O(21)-C(71)-C(21)	119.8(4)
O(21)-C(71)-O(31)	127.6(4)	O(31)-C(81)-C(91)	107.1(4)
		N(21)-C(101)-C(41)	174.7(5)
C(52)-N(12)-C(12)	124.6(4)	H(12)-N(12)-C(12)	119(2)
H(12)-N(12)-C(52)	116(2)	C(72)-O(32)-C(82)	115.5(4)
C(12)-C(22)-C(72)	129.7(4)	C(32)-C(22)-C(72)	112.0(4)
C(32)-C(22)-C(12)	118.3(4)	C(22)-C(32)-C(42)	118.1(4)
C(22)-C(32)-H(32)	115(2)	H(32)-C(32)-C(42)	126(2)
C(32)-C(42)-C(102)	118.4(4)	C(32)-C(42)-C(52)	121.7(4)
C(52)-C(42)-C(102)	119.9(4)	O(12)-C(52)-C(42)	123.3(5)
N(12)-C(52)-C(42)	116.4(4)	N(12)-C(52)-O(12)	120.3(4)
N(12)-C(12)-C(22)	120.9(4)	C(22)-C(12)-C(62)	126.5(4)
N(12)-C(12)-C(62)	112.6(4)		
		O(32)-C(72)-C(22)	115.5(4)
O(22)-C(72)-C(22)	126.5(4)	O(22)-C(72)-O(32)	117.8(4)
O(32)-C(82)-C(92)	104.3(5)		
N(22)-C(102)-C(42)	173.9(5)	C(13)-N(13)-C(53)	127.4(4)
H(13)-N(13)-C(53)	120(4)	H(13)-N(13)-C(13)	112(4)
C(73)-O(33)-C(83)	120.3(4)	N(13)-C(13)-C(63)	113.3(4)
N(13)-C(13)-C(23)	115.8(5)	C(23)-C(13)-C(63)	130.7(5)
C(13)-C(23)-C(73)	124.8(4)	C(13)-C(23)-C(33)	119.5(4)
C(33)-C(23)-C(73)	115.7(4)	C(23)-C(33)-C(43)	123.7(4)
C(33)-C(43)-C(103)	124.7(4)	C(33)-C(43)-C(53)	119.2(4)
C(53)-C(43)-C(103)	115.8(4)	O(13)-C(53)-C(43)	123.9(5)
N(13)-C(53)-C(43)	114.2(5)	N(13)-C(53)-O(13)	121.9(4)
O(33)-C(73)-C(23)	116.9(4)	O(23)-C(73)-C(23)	125.1(5)
O(23)-C(73)-O(33)	117.9(5)	O(33)-C(83)-C(93)	107.4(5)
N(23)-C(103)-C(43)	176.4(5)		

The packing of the dimers, which were centrosymmetrically related for space group conditions, do not bear any significant interaction among them being separated by normal Van der Waals distances. The unit cell content is shown in Figure 4. The bulky *t*-butyl substituent does not allow molecular stacking.

Ester ***2f.*** This compound differed from the previous ones by the presence of the phenyl instead of the methyl or *t*-butyl group. The asymmetric unit in the centrosymmetric triclinic cell was a single molecule.

However, also in this case there was the formation of dimers between the two centrosymmetrically related molecules present in the cell which were connected *via* hydrogen bonds: N(1)...O(1) was 2.795(6) Å and N(1)−H(1)...O(1) 1.94(7) Å, whereas the angle N(1)−H(1)...O(1) was 171.5(5)°.

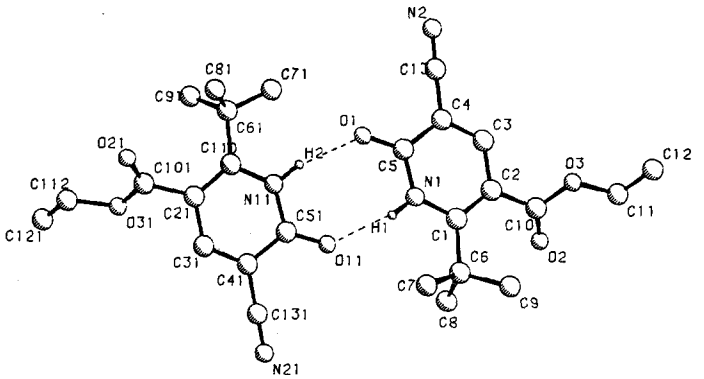


Fig. 3. Perspective view of the dimer in the 2-*t*-butyl derivative **(2e)**.

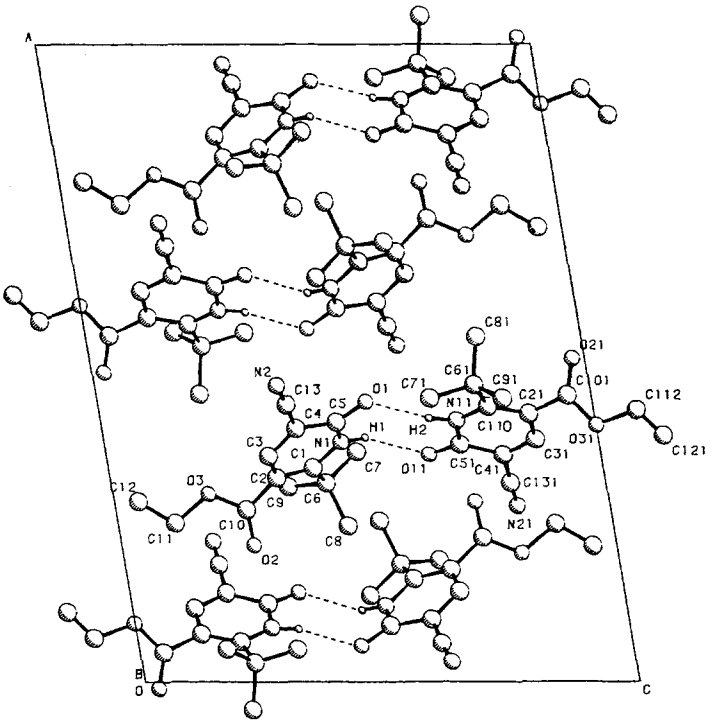


Fig. 4. Unit cell content of the 2-*t*-butyl derivative **(2e)**.

Table V. Selected interatomic bonds and angles for the 2-*t*-butyl derivative **(2e)**.

a) Selected bond distances (Å) for 2e .

N(1)-H(1)	0.86(6)	N(1)-C(1)	1.358(7)
N(1)-C(5)	1.388(5)	N(2)-C(15)	1.134(5)
O(1)-C(5)	1.225(7)	O(2)-C(12)	1.185(7)
O(3)-C(12)	1.329(4)	O(3)-C(13)	1.452(9)
C(1)-C(2)	1.385(7)	C(1)-C(6)	1.482(5)
C(2)-C(3)	1.411(5)	C(2)-C(12)	1.480(8)
C(3)-C(4)	1.361(8)		
C(4)-C(5)	1.430(7)	C(4)-C(15)	1.447(5)
C(6)-C(7)	1.387(7)	C(6)-C(11)	1.381(7)
C(7)-C(8)	1.388(6)	C(8)-C(9)	1.375(8)
C(9)-C(10)	1.364(8)		
C(10)-C(11)	1.379(6)		
C(13)-C(14)	1.477(8)		

b) Selected bond angles (°) for 2e .

C(1)-N(1)-C(5)	127.2(4)	H(1)-N(1)-C(5)	113(3)
H(1)-N(1)-C(1)	120(3)	C(12)-O(3)-C(13)	117.0(4)
N(1)-C(1)-C(6)	114.8(4)	N(1)-C(1)-C(2)	118.0(4)
C(2)-C(1)-C(6)	127.1(4)	C(1)-C(2)-C(12)	126.6(4)
C(1)-C(2)-C(3)	118.3(4)	C(3)-C(2)-C(12)	115.1(4)
C(2)-C(3)-C(4)	121.5(4)		
		C(3)-C(4)-C(15)	121.4(4)
C(3)-C(4)-C(5)	121.6(4)	C(5)-C(4)-C(15)	117.0(4)
O(1)-C(5)-C(4)	125.6(4)	N(1)-C(5)-C(4)	113.3(4)
N(1)-C(5)-O(1)	121.0(4)	C(1)-C(6)-C(11)	119.8(4)
C(1)-C(6)-C(7)	120.7(4)	C(7)-C(6)-C(11)	119.5(4)
C(6)-C(7)-C(8)	119.3(5)		
		C(7)-C(8)-C(9)	120.2(5)
C(8)-C(9)-C(10)	120.7(6)		
		C(9)-C(10)-C(11)	119.5(5)
C(6)-C(11)-C(10)	120.8(5)		
		O(3)-C(12)-C(2)	114.0(4)
O(2)-C(12)-C(2)	123.3(5)	O(2)-C(12)-O(3)	122.8(4)
O(3)-C(13)-C(14)	107.2(5)		
N(2)-C(15)-C(4)	179.4(5)		

Significant bond distances and angles are reported in Table VI. The molecular structure is shown in Figure 5 with the atom labelling; the packing diagram evidencing the dimer molecules is in Figure 6.

The molecular conformation was characterized by a planar pyridone ring from which the ester moiety is twisted by 18° and the phenyl group by 52°. No molecular stackings were present in the structure; the dimers were separated by normal Van der Waals interactions.

Results

The effects of milrinone analogues, namely esters **2a–g**, carboxylic acids **3a–g** and nitriles **4a,d,f,g** were tested on

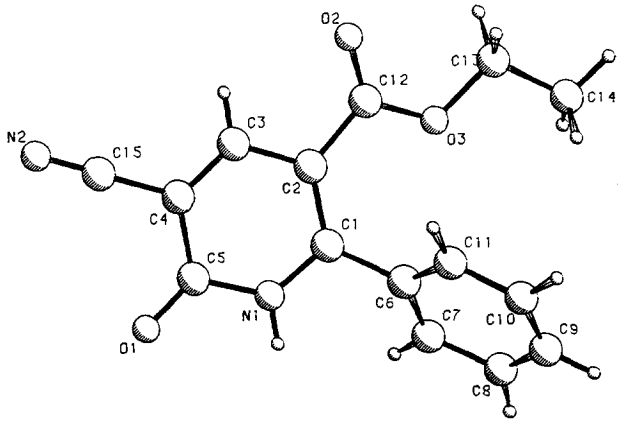


Fig. 5. Perspective view of a molecule of the 2-phenyl derivative **(2f)**.

Table VI. Selected bond distances and angles for the 2-phenyl derivatives **(2f)**.

a) Selected bond distances (Å) for 2f.

N(1)-H(1)	0.79(5)	N(11)-H(2)	0.87(6)
N(1)-C(1)	1.361(6)	N(1)-C(5)	1.394(7)
O(1)-C(5)	1.227(6)	O(2)-C(10)	1.199(6)
C(1)-C(2)	1.373(7)	C(1)-C(6)	1.536(7)
C(2)-C(3)	1.408(7)	C(2)-C(10)	1.487(6)
C(3)-C(4)	1.367(7)	C(4)-C(5)	1.431(7)
C(4)-C(13)	1.444(8)	C(6)-C(7)	1.532(7)
C(6)-C(8)	1.530(8)	C(6)-C(9)	1.535(8)
C(10)-O(3)	1.332(7)	O(3)-C(11)	1.458(6)
C(11)-C(12)	1.37(1)	C(13)-N(2)	1.124(8)
N(11)-C(110)	1.358(6)	N(11)-C(51)	1.385(7)
O(11)-C(51)	1.223(6)	O(21)-C(101)	1.193(8)
C(110)-C(21)	1.384(7)	C(110)-C(61)	1.526(7)
C(21)-C(31)	1.398(8)	C(21)-C(101)	1.494(7)
C(31)-C(41)	1.361(7)	C(41)-C(51)	1.444(7)
C(41)-C(131)	1.437(8)	C(61)-C(71)	1.504(8)
C(101)-O(31)	1.330(8)	N(21)-C(131)	1.137(9)
O(31)-C(111)	1.46(1)	C(111)-C(121)	1.51(2)

b) Selected bond angles (°) for 2f.

H(1)-N(1)-C(1)	116(4)	H(1)-N(1)-C(5)	116(4)
C(1)-N(1)-C(5)	127.8(4)	N(1)-C(1)-C(6)	115.5(4)
N(1)-C(1)-C(2)	117.1(4)	C(2)-C(1)-C(6)	127.3(4)
C(1)-C(2)-C(10)	124.5(5)	C(1)-C(2)-C(3)	118.5(5)
C(3)-C(2)-C(10)	116.9(4)	C(2)-C(3)-C(4)	122.6(5)
C(3)-C(4)-C(13)	122.0(5)	C(3)-C(4)-C(5)	120.0(5)
C(5)-C(4)-C(13)	118.0(5)	O(1)-C(5)-C(4)	126.8(5)
N(1)-C(5)-C(4)	113.1(4)	N(1)-C(5)-O(1)	120.1(5)
C(1)-C(6)-C(9)	111.4(4)	C(1)-C(6)-C(8)	108.5(4)
C(1)-C(6)-C(7)	110.6(4)	C(8)-C(6)-C(9)	110.5(5)
C(7)-C(6)-C(9)	106.7(4)	C(7)-C(6)-C(8)	109.2(5)
O(2)-C(10)-C(2)	125.9(5)	C(2)-C(10)-O(3)	110.3(4)
O(2)-C(10)-O(3)	123.8(5)	C(10)-O(3)-C(11)	116.0(4)
O(3)-C(11)-C(12)	109.8(6)	C(4)-C(13)-N(2)	177.6(6)
H(2)-N(11)-C(110)	117(3)	H(2)-N(11)-C(51)	115(3)
C(110)-N(11)-C(51)	128.2(4)	N(11)-C(110)-C(61)	116.6(4)
N(11)-C(110)-C(21)	116.2(5)	C(21)-C(110)-C(61)	127.1(5)
C(110)-C(21)-C(101)	124.8(5)	C(110)-C(21)-C(31)	119.7(5)
C(31)-C(21)-C(101)	115.5(5)	C(21)-C(31)-C(41)	122.0(5)
C(31)-C(41)-C(131)	121.8(5)	C(31)-C(41)-C(51)	120.4(5)
C(51)-C(41)-C(131)	117.8(5)	O(11)-C(51)-C(41)	125.2(5)
N(11)-C(51)-C(41)	113.1(4)	N(11)-C(51)-O(11)	121.7(5)
C(110)-C(61)-C(71)	111.4(5)	O(21)-C(101)-C(21)	125.8(6)
C(21)-C(101)-O(31)	110.9(5)	O(21)-C(101)-O(31)	123.2(5)
C(41)-C(131)-N(21)	178.2(7)	C(101)-O(31)-C(111)	116.1(6)
O(31)-C(111)-C(121)	112.5(9)		

contractile activity and frequency rate of spontaneously beating atria from reserpine-treated guinea-pigs and were compared with the effects of amrinone and milrinone on the same parameters of cardiac activity. Among the above compounds, ester **2a** induced the greatest increase of contractile force. As reported in Table VIII the maximum inotropic effect was evoked by a concentration of **2a** (10^{-4} M) which was lower than that of milrinone ($5 \cdot 10^{-4}$ M) and this maximum, expressed as percent variation from the control, was greater than that of milrinone. The positive inotropic effect was followed by a negative phase of inotropism that was more pronounced than that induced by milrinone, but completely reversible by washing the heart preparation. The action on the atria was also characterized by an increase of frequency rate quite similar to that induced by milrinone (Table IX).

Also ester **2b** (Tables VIII and IX) and nitrile **4a** (Tables X and XI) were positive inotropic and chronotropic agents though their influence on inotropism and chronotropism was similar or even less marked in comparison with that induced by milrinone. Interestingly, esters **2e** and **2g** (Table VIII) induced a marked negative inotropic effect and a negative influence on chronotropism (Table IX). Both these negative effects were completely reversed by washing the preparations with drug-free medium. Ester

2c, acids **3a**, **3c**, **3e** and nitrile **4d** were scarcely active as inotropic agents (Tables VIII–X) and, with the exception of **2c**, the contractile effect was characterized by a concomitant reduction in frequency rate (Tables IX–XI). Acid **3b** and nitrile **4g** were less active than esters **2e** or **2g** as negative inotropic and chronotropic agents (Tables X and XI), whereas acids **3f**, **3g** and nitrile **4f** had no relevant influence on contractility or frequency rate of the atria (Tables X and XI). The inotropic property of the most active compound, the ester **2a**, was also confirmed in the electrically driven left atria (Table XII), where the regularly induced pulses do not influence the contractile response to the tested drugs. Figure 7 gives the comparison between esters **2a**, **2b** and the more active inotropic agent, isoprenaline.

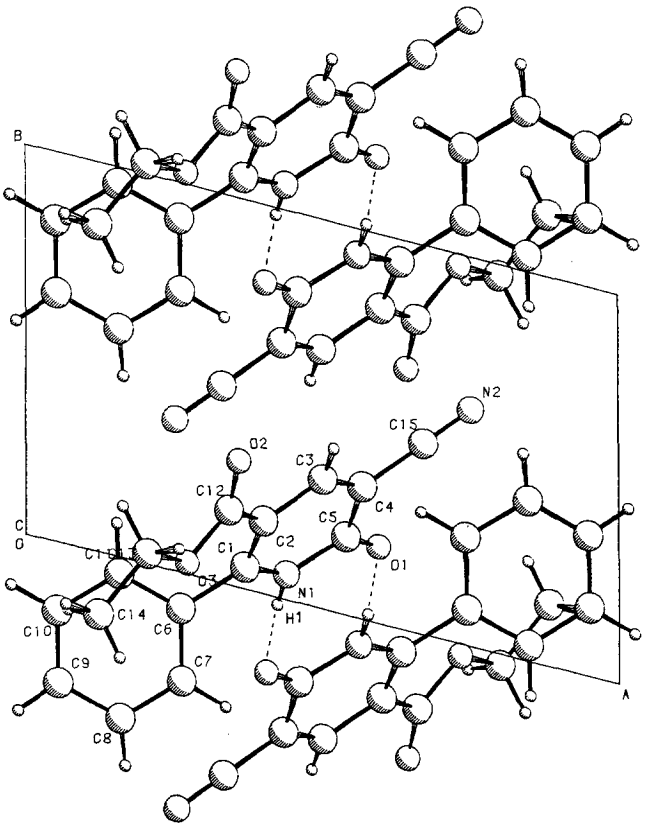


Fig. 6. Crystal packing of the 2-phenyl derivative (**2f**).

Consequently, the 3 structurally characterized compounds have been chosen as significant examples of the cardiotonic activity: ester **2a** with the most positive, ester **2e** with the most negative and ester **2f** with a weak positive inotropic activity. Their conformation in the solid state indicates the following: ester **2a** seemed to satisfy all the requirements indicated in the five-point model for positive inotropic activity [15, 16]: the presence of a strong dipole, the CO group; the presence of the adjacent acid proton of the NH group; a methyl-sized lipophilic space; a relatively flat overall topography, the ester group being practically coplanar with the pyridone ring (the presence of 4 independent molecules in the triclinic cell, differing slightly by the twisting of the ester moieties, suggests that these conformations are among the more favourable energetically and in any case they can be considered as good indicators of probable low energy conformations); finally, a hydrogen bond acceptor site opposite the dipole, which in these molecules is represented by the carbonyl oxygen of the ester group, always free from interactions with adjacent molecules.

The intermolecular hydrogen bonds are always between the N proton and the ring CO oxygen with formation of dimers; the strength of these interactions was the greatest when compared with those of the other examined compounds (Table VII) (the average distance N(donor)...O is 2.780(5) Å *vs* 2.795(6) Å for the scarcely active ester **2f** and 2.888(6) Å for ester **2e** with reverse action: negative inotropic). Moreover, comparison of the H...O distances in ester **2a**, particularly for the II and III molecules, indicated a delocalization of the N proton in the direction of the O acceptor. This behaviour was peculiar to the ester **2a**, as well as the presence of π interactions between dimers.

The radical change in activity which was presented by ester **2e**, the *t*-butyl substituted ester, could be related to

Table VII. Comparison of the hydrogen bond interactions in the examined compounds (**2a, 2e, 2f**) having different pharmacological activities.

	Pharm. activity	Donor-H (Å)		Donor...Acceptor (Å)		H...Acceptor (Å)		DonorH...Acceptor (°)		
2a	Positive inotropic	N(1)-H(1)	0.86(5)	N(1)...O(13)	2.779(4)	H(1)....O(13)	1.94(5)	164(4)	(I)	dimer
		N(13)-H(13)	0.76(4)	N(13)..O(1)	2.778(5)	H(13)...O(1)	2.04(5)	163(5)	(IV)	
		N(11)-H(11)	1.08(4)	N(11)..O(12)	2.801(4)	H(11)...O(12)	1.73(4)	171(4)	(II)	dimer
		N(12)-H(121)	1.16(4)	N(12)..O(11)	2.760(4)	H(121)..O(11)	1.61(4)	169(4)	(III)	
2e	Negative inotropic	N(1)-H(1)	0.81(4)	N(1)....O(11)	2.839(6)	H(1)....O(11)	2.17(4)	139(4)		dimer
		N(11)-H(11)	0.95(6)	N(11)...O(11)	2.938(5)	H(11)...O(1)	2.03(7)	160(5)		
2f	scarcely active inotropic	N(1)-H(1)	0.86(6)	N(1)....O(1)	2.795(6)	H(1)....O(1)	1.94(7)	171.5(5)		half dimer

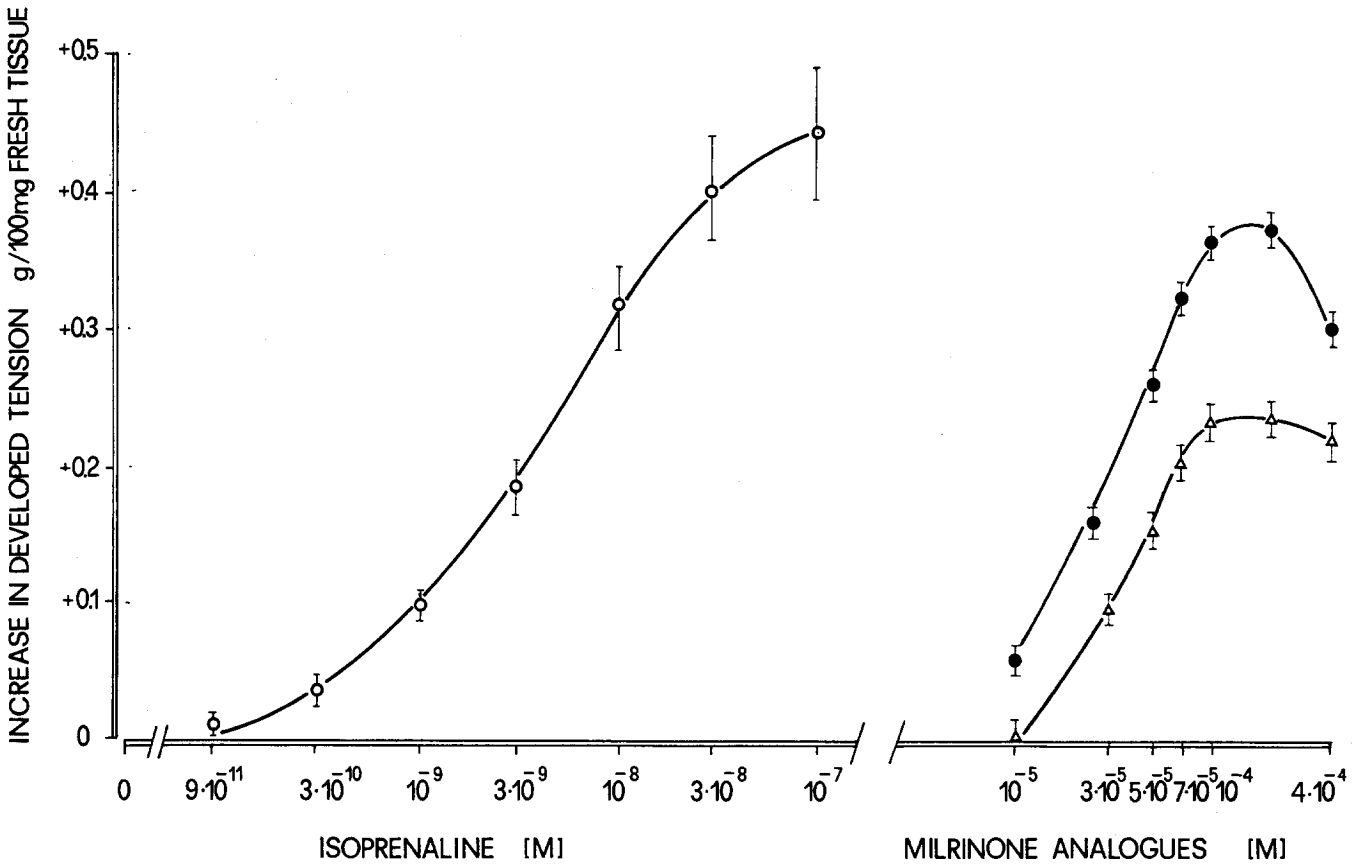


Fig. 7. Comparison between the inotropic effect induced by isoprenaline (○—○) and the milrinone analogues **2a** (●—●) and **2b** (△—△). The compounds were added cumulatively to the perfusion medium of spontaneously beating atria from reserpine-treated guinea pigs. Each data is mean ± SEM of 10 determinations from different experiments.

Table VIII. Effects of esters **2a–g** on contractile force of spontaneously beating atria from reserpine-treated guinea pigs: comparison with amrinone and milrinone.

Comp.	DEVELOPED TENSION (% increase over control)					
	10^{-5}M	5.10^{-5}M	10^{-4}M	5.10^{-4}M	10^{-3}M	2.10^{-3}M
Amrinone	0.52 ± 0.01	4.50 ± 0.41	10.22 ± 0.85	19.03 ± 0.72	23.12 ± 1.42	19.07 ± 0.79
Milrinone	7.43 ± 0.61	30.12 ± 2.33	46.42 ± 0.81	48.32 ± 4.18	45.12 ± 3.21	30.21 ± 2.98
2a	26.34 ± 1.01	58.32 ± 3.42	63.48 ± 4.61	54.38 ± 5.11	27.41 ± 2.01	-10.21 ± 1.92
2b	16.21 ± 1.31	34.41 ± 2.01	43.19 ± 3.48	42.22 ± 3.59	-59.2 ± 3.30	-73.81 ± 2.92
2c	6.51 ± 0.71	18.21 ± 1.51	20.03 ± 2.03	7.51 ± 2.48	-23.00 ± 1.40	-44.87 ± 6.66
2d	3.50 ± 0.91	10.32 ± 0.98	13.41 ± 1.21	5.61 ± 0.75	-58.58 ± 3.43	-86.29 ± 1.34
2e	5.11 ± 0.69	-32.61 ± 2.48	-45.72 ± 4.51	-67.31 ± 7.31	-73.21 ± 8.48	-75.03 ± 6.52
2f	1.12 ± 0.90	2.91 ± 2.21	8.33 ± 2.35	2.51 ± 1.41	- 1.56 ± 0.91	-16.55 ± 1.94
2g	8.42 ± 2.22	- 2.51 ± 1.00	-20.18 ± 1.98	-57.12 ± 4.61	-66.7 ± 3.11	-80.42 ± 3.41

The compounds were added cumulatively to the perfusion medium of the atria. Each data is mean ± SEM of 10 determinations from 10 different experiments. (−) = negative inotropic effect.

Table IX. Effect of esters **2a–g** on frequency rate of spontaneously beating atria from reserpine-treated guinea pigs: comparison with amrinone and milrinone.

Comp.	FREQUENCY RATE (% increase over control)					
	10^{-5}M	5.10^{-5}M	10^{-4}M	5.10^{-4}M	10^{-3}M	2.10^{-3}M
Amrinone	0.00 ± 0.00	1.50 ± 0.50	6.25 ± 1.35	7.20 ± 1.46	9.87 ± 0.46	12.25 ± 1.56
Milrinone	6.57 ± 1.25	12.5 ± 1.25	21.00 ± 1.00	23.00 ± 1.91	24.25 ± 1.84	30.25 ± 1.65
2a	2.08 ± 0.83	9.58 ± 0.86	15.50 ± 1.08	25.22 ± 0.84	26.40 ± 2.60	31.42 ± 1.42
2b	- 2.58 ± 1.95	- 3.20 ± 0.83	6.3 ± 0.39	20.58 ± 0.50	30.25 ± 1.37	22.12 ± 1.16
2c	5.77 ± 1.33	7.52 ± 1.05	9.00 ± 0.69	11.64 ± 1.42	29.36 ± 2.84	34.28 ± 1.12
2d	-11.22 ± 1.01	- 5.97 ± 0.67	- 7.92 ± 1.18	- 5.14 ± 0.63	- 6.68 ± 0.94	-10.70 ± 1.27
2e	0.00 ± 0.00	- 5.43 ± 0.63	- 6.01 ± 0.31	- 9.67 ± 0.18	-16.33 ± 1.20	-19.33 ± 2.77
2f	- 6.78 ± 0.91	-10.71 ± 0.83	-14.11 ± 0.76	-13.90 ± 1.55	-17.50 ± 1.40	-15.62 ± 0.90
2g	0.00 ± 0.00	- 0.70 ± 0.15	- 5.00 ± 1.15	-15.50 ± 0.29	-24.50 ± 1.32	-36.5 ± 2.59

The compounds were added cumulatively to the perfusion medium of the atria. Each data is mean ± SEM of 10 determinations from 10 different experiments. (−) = negative chronotropic effect.

Table X. Effect of acids **3a–g** and nitriles **4a,d,f,g** on contractile force of spontaneously beating atria from reserpine-treated guinea pigs: comparison with ester **2a**.

Comp.	DEVELOPED TENSION (% increase over control)					
	10^{-5}M	5.10^{-5}M	10^{-4}M	5.10^{-4}M	10^{-3}M	2.10^{-3}M
2a	16.06 ± 1.02	36.60 ± 1.33	73.24 ± 3.07	63.05 ± 1.41	52.25 ± 2.59	-13.25 ± 1.26
3a	2.21 ± 0.15	2.28 ± 0.18	11.93 ± 0.96	11.78 ± 0.96	10.92 ± 0.50	-16.75 ± 0.54
3b	3.38 ± 0.20	-33.25 ± 1.50	-36.85 ± 0.81	-34.62 ± 2.33	-33.74 ± 1.91	-36.21 ± 1.30
3c	1.16 ± 0.48	2.25 ± 0.92	6.90 ± 0.41	7.50 ± 0.55	11.37 ± 0.58	18.98 ± 0.79
3d	4.57 ± 0.54	8.52 ± 0.61	10.50 ± 0.86	11.35 ± 0.61	12.62 ± 0.35	14.60 ± 0.61
3e	-1.9 ± 0.26	1.75 ± 0.31	5.95 ± 0.57	3.07 ± 0.26	4.57 ± 0.61	8.95 ± 0.27
3f	0.84 ± 0.31	0.14 ± 0.05	1.31 ± 0.66	0.24 ± 0.12	0.67 ± 0.34	1.17 ± 0.59
3g	3.00 ± 0.08	2.52 ± 0.32	2.97 ± 0.40	3.10 ± 0.22	2.20 ± 0.30	2.27 ± 0.48
4a	3.40 ± 0.12	7.17 ± 0.47	13.16 ± 0.55	24.55 ± 2.07	34.20 ± 1.49	44.65 ± 2.50
4d	-1.31 ± 0.25	-1.13 ± 0.10	2.57 ± 0.14	5.64 ± 0.44	20.71 ± 0.88	22.73 ± 0.79
4f	0.60 ± 0.18	1.65 ± 0.06	3.23 ± 0.34	3.40 ± 0.30	2.91 ± 0.33	3.32 ± 0.19
4g	0.30 ± 0.15	2.92 ± 0.33	-7.22 ± 0.50	-20.23 ± 1.67	-34.14 ± 1.36	-46.42 ± 4.13

The compounds were added cumulatively to the perfusion medium of the atria. Each data is mean ± SEM of 6 determinations from 6 different experiments. (−) = negative inotropic effect.

the following conformational changes: the bulky *t*-butyl group cannot be accommodated in the small hydrophobic pocket of the methyl group, but in some other larger cavity of the receptor causing the reverse action, and in addition a large rotation of the ester moiety with respect to the pyridone ring existed (49.5° and 131.9° are the values of the dihedral angles in the 2 independent molecules). All this is accompanied by a lack of an overall molecular planarity. The hydrogen bond interactions were weaker (Table VII) and the molecular stacking was missing.

The third derivative **2f** was scarcely active and the only requirement of the five-point model which was not satisfied seemed to be related to the presence of the bulkier hydrophobic phenyl moiety.

In fact, apart from this group, the overall geometry was rather flat (the ester moiety is rotated just 18° with respect

Table XI. Effect of acids **3a–g** and nitriles **4a,d,f,g** on frequency rate of spontaneously beating atria from reserpine-treated guinea-pigs: comparison with ester **2a**.

Comp	FREQUENCY RATE (% increase over control)					
	10^{-5}M	5.10^{-5}M	10^{-4}M	5.10^{-4}M	10^{-3}M	2.10^{-3}M
2a	4.05 ± 0.42	11.80 ± 1.76	27.50 ± 2.90	32.40 ± 9.86	40.77 ± 3.91	48.92 + 2.25
3a	- 2.79 ± 0.36	- 4.40 ± 0.19	- 4.79 ± .62	- 5.67 ± 0.34	- 7.35 ± 0.88	-11.85 ± 1.94
3b	- 6.32 ± 0.49	- 9.10 ± 0.57	-14.79 ± 1.20	-16.98 ± 1.11	-16.37 ± 0.91	-16.39 ± 0.39
3c	- 2.97 ± 0.48	- 5.32 ± 0.44	- 6.38 ± 1.33	- 7.14 ± 0.50	- 1.01 ± 0.18	-13.72 ± 1.24
3d	- 4.80 ± 0.81	- 7.68 ± 0.99	-10.42 ± 1.12	-10.4 ± 1.07	-13.05 ± 0.85	-14.11 ± 1.12
3e	- 3.05 ± 0.56	- 2.64 ± 0.56	- 2.27 ± 0.49	- 3.30 ± 0.43	- 5.70 ± 1.08	-11.37 ± 0.99
3f	- 0.54 ± 0.16	- 1.57 ± 0.54	- 2.03 ± 0.47	- 3.87 ± 0.54	- 7.47 ± 0.76	- 7.67 ± 0.83
3g	- 2.62 ± 1.14	- 4.12 ± 0.24	- 5.47 ± 0.71	- 8.91 ± 1.22	-11.38 ± 1.01	-15.53 ± 1.79
4a	0.39 ± 0.08	0.27 ± 0.08	0.79 ± 0.12	1.06 ± 0.13	6.72 ± 1.08	7.37 ± 1.04
4d	3.60 ± 0.33	- 3.40 ± 0.21	- 3.80 ± 0.19	- 4.47 ± 1.01	- 2.10 ± 0.55	- 2.40 ± 0.47
4f	- 2.04 ± 0.65	- 5.62 ± 0.98	- 6.40 ± 1.10	- 9.35 ± 1.02	- 9.82 ± 0.81	-11.35 ± 1.77
4g	-11.14 ± 1.88	-11.87 ± 1.05	-12.19 ± 1.85	-16.10 ± 0.81	26.63 ± 3.10	-30.15 ± 2.32

The compounds were added cumulatively to the perfusion medium of the atria. Each data is mean ± SEM of 6 determinations from 6 different experiments. (−) = negative inotropic effect.

Table XII. Effect of esters **2a** and **2b** on electrically driven left atria from reserpine-treated guinea-pigs: comparison with milrinone

(M)	DEVELOPED TENSION (g/100 mg fresh tissue)					
	Milrinone	% increase	2a	% increase	2b	%increase
-	0.59±0.03		0.44±0.04		0.44±0.03	
10^{-5}	0.67±0.05	13 (n.s.)	0.57±0.05	29 (P<0.05)	0.51±0.04	16 (n.s.)
5.10^{-5}	0.73±0.05	23 (P<0.05)	0.66±0.05	50 (P<0.001)	0.59±0.05	34 (P<0.05)
10^{-4}	0.82±0.05	38 (P<0.01)	0.72±0.04	63 (P<0.001)	0.63±0.04	43 (P<0.00)
5.10^{-4}	0.88±0.03	49 (P<0.005)	0.56±0.06	27 (P<0.05)	0.64±0.06	45 (P<0.00)
10^{-3}	0.85±0.02	44 (P<0.005)	0.49±0.05	11 (n.s.)	0.44±0.09	- (n.s.)

Milrinone, **2a** and **2b** were added cumulatively. Each data is mean ± SEM of 7 to 11 assays in different experiments. The statistical significance of the differences from controls (atria incubated without inotropic agents) was calculated by the Student's *t*-test.

to the pyridone ring) and the H...O bond interaction is midway between the values reported for **2a** and **2e**. However, the phenyl group is rotated by 52° with respect to the pyridone plane, thus destroying the "quasi" planarity of the system without producing stacking of the molecules. It appears, therefore, that the zone which seems to affect the biological properties of the molecules is related mainly to the 2 position. The bulk of the substituent can diminish the positive inotropic activity until in the extreme case it can reverse the action and an equally important effect can influence the molecular geometry.

In conclusion, from the present data the ethyl ester **2a** (R = CH_3) appears to be the most promising cardiotonic compound, whereas among the esters the activity decreased until faded or even reversed by increasing or branching the chain R. Moreover, negative inotropic agents like esters **2e** and **2g** may be useful to understand the relationship between chemical structure and mechanism of action of these analogues of milrinone.

Experimental protocols

Chemistry

Melting points were determined with a Fisher-Johns apparatus and are uncorrected. UV spectra were measured in 95% ethanol with a Perkin–Elmer Lambda 3 spectrophotometer. IR spectra were taken on a Perkin–Elmer 398 spectrophotometer, and ^1H NMR spectra were recorded on a Perkin–Elmer R–600 instrument (60 MHz, TMS as internal standard, *J* in Hz).

Analyses for C,H,N were within ± 0.3% of the theoretical values.

*General procedure for esters of 2-substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acids **2a–g***

Ethyl or methyl 2-dimethylaminomethylene-3-oxoalkanoates **1a–g** [5] (20 mmol), dissolved in anhydrous ethanol (20–200 ml), were added at room temperature to a solution of sodium cyanoacetamide in anhydrous ethanol which had been prepared by adding a solution of sodium ethoxide (from sodium (0.46 g, 20 mmol) and anhydrous ethanol (20 ml)) to cyanoacetamide (1.68 g, 20 mmol) dissolved in warm anhydrous ethanol (20 ml). The mixture was stirred at room temperature for 24 h (48 h in the case of **2e**). The precipitate was then filtered, washed thoroughly with anhydrous diethyl ether and dissolved in the minimum amount of water. In the case of **2b**, **2d**, **2e** and **2f** it was necessary to evaporate the filtered ethanol solution in order to obtain a further crop of product, that was in certain cases the main portion. The aqueous solution was filtered and acidified with 6 N HCl (pH ≈ 1). The precipitate was filtered, washed with water, dried in an oven at 100°C and recrystallized from 95% ethanol.

*General procedure for 2-substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acids **3a–g***

Potassium hydroxide (1.68 g, 30 mmol) dissolved in 95% ethanol (20 ml) was added to a solution of **2a–g** (10 mmol) in the same solvent (20 ml). The resulting solution was refluxed with stirring (see Table II for time periods). The solvent was then evaporated under reduced pressure and the residue was dissolved with water (50 ml). The aqueous solution was acidified with 6 N HCl (pH ≈ 1), the precipitate filtered, washed with water and recrystallized from 95% ethanol.

Acid **3e** was obtained as a mixture with the starting ester even after a very prolonged reaction time (Table II). Separation was achieved by treating the mixture in a separating funnel with saturated $NaHCO_3$ solution and chloroform followed by acidification of the alkaline solution as above.

Acid **3a** retained a molecule of water which we were unable to remove either by repeated recrystallizations from anhydrous ethanol, or by drying in an oven at 100°C under reduced pressure.

*General procedure for 6-substituted 1,2-dihydro-2-oxo-3-pyridinecarbonitriles **4a–g***

A solution of carboxylic acid **3a–g** (10 mmol) in quinoline (25 ml) containing copper powder (0.22 g) was refluxed (see Table III for time periods). The reaction mixture was filtered when hot and chloroform (100 ml) was added to the cooled filtrate. The solution was extracted twice with 6 N HCl (50 ml each time), washed with water and dried ($MgSO_4$). The solid obtained after evaporation of the solvent was recrystallized from a suitable solvent (see Table III).

Pharmacological methods

Isolated atria preparations

Reserpine-treated male guinea-pigs (300–500 g) were used. Reserpine 2 mg/kg^{-1} i.p. was given 48 and 12 h before the experiments in order to eliminate the influence of noradrenaline which might be released from sympathetic nerve terminals [17].

The animals were killed by cervical dislocation followed by exsanguination and the atria were separated from the ventricles and suspended vertically in a bath containing 30 ml of physiological salt solution of the following composition (mmol·l^{-1}): NaCl, 120; KCl, 2.7; $MgCl_2$, 0.9; NaH_2PO_4, 0.4; $CaCl_2$, 1.37; $NaHCO_3$, 11.9; glucose, 5.5. The solution was maintained at 29°C and was bubbled vigorously with a mixture of 95% O_2 and 5% CO_2 which produced a pH of 7.5. Resting tension was adjusted at 1.0 g and registered by a writing oscillograph (Basile, Unirecord system, Model 7050). The basal developed tension ranged from 0.8 to 1.3 mN. Left atria were mounted on punctate electrodes with a load of 0.5 g and stimulated at a frequency of 1.5 Hz by square wave electrical pulses of 3 ms duration and a voltage 10–20% greater than threshold delivered by a Grass stimulator (Model 24KR). The basal developed tension ranged from 0.09–0.20 mN.

Inotropic activity

The experiments were performed on atria obtained from reserpine-treated animals. Reserpine (2 mg kg^{-1}, i.p.) was given 48 and 12 h before the animals were killed in order to eliminate the influence of noradrenaline which might be released from sympathetic nerve terminals [17]. Noradrenaline depletion was checked by exposing isolated atria to a single dose of tyramine (2 μg·ml^{-1}) before starting the experiments. The drugs were added to the perfusion fluid after a 90 min equilibration period. All the compounds were added cumulatively and the inotropic effect was recorded for 5 min after it reached maximum before washing the preparations or before adding a higher concentration. The inotropic effect was determined both on spontaneously beating atria and electrically driven left atrium.

Crystal data

Ester **2a** $C_{10}H_{10}N_2O_3$, M = 206.2 Triclinic, a = 12.240(3), b = 7.869(2), c = 11.562(3) Å, α = 96.11(4)°, β = 101.27(4)°, γ = 110.73(4)°; V = 1002.5 Å^3, Z = 4, $D_{calcd.}$ = 1.366 g·cm^{-3}, μ(MoK_α radiation, λ = 0.7107 Å) = 0.64 cm^{-1}, space group P1 or P$\bar{1}$ (P1 was confirmed after structure determination).

Ester **2e** $C_{13}H_{16}N_2O_3$, M = 248.3. Monoclinic, a = 20.262(5), b = 8.665(3), c = 15.810(4) Å, β = 99.76(3)°, V = 2735.6 Å^3, Z = 8, $D_{calcd.}$ = 1.208 g·cm^{-3}, μ(MoK_α radiation, λ = 0.7107 Å) = 0.52 cm^{-1}, space group P21/a, uniquely from systematic absences OkO when k ≠ 2n, hO1 when h ≠ 2n).

Ester **2f** $C_{15}H_{12}N_2O_3$, M = 268.3. Triclinic, a = 12.385(3), b = 8.071(2), c = 7.871(2) Å, α = 65.47(3)°, β = 106.35(3)°, γ = 109.02(4)°, V = 666.8 Å^3, Z = 4, $D_{calcd.}$ = 1.336 g·cm^{-3}, μ(MoK_α radiation, λ = 0.7107 Å) = 0.57 cm^{-1}, space groups P1 or P$\bar{1}$ (P$\bar{1}$ was confirmed after structure determination).

Crystallographic measurements

For ester **2a** the intensity data (±h, ±k, 1) were measured on a Philips PW 1100 automated diffractometer using MoK_α radiation (λ = 0.7107 Å) by ν–2 ν scan technique, 2 ν_{max} = 50°. From totals of 3741 after averaging of equivalents, those 2263 with I > 3 σ(I) were retained for the structure analysis. For ester **2e** the intensity data (±h, k, 1) were measured as for **2a** (2 ν_{max} = 50°). From totals of 5236 after averaging of equivalents, those 2369 with I > 3 σ(I) were used for the structure analysis. For ester **2f** the intensity data (±h, ±k, 1) were measured as for **2a** (2 ν_{max} = 50°). From totals of 2541 after averaging of equivalents, those 1141 with I > 3 σ(I) were used for the structure analysis.

All the intensity data were corrected for the usual Lorentz and polarization effects. Refined unit cell parameters for each compound were derived from the diffractometer setting angles for 25 high-order reflections.

X-ray structure analyses

The 3 crystal structures were solved by using direct method programs MULTAN [18] and SHELX 76 [19]. Approximate non-hydrogen atom coordinates were obtained from E maps. Hydrogen atoms were located in difference Fourier syntheses evaluated at late stages in the analyses. Full-matrix least-squares refinement of non-hydrogen atom positional and anisotropic thermal parameters with hydrogen atoms refined with a unique fixed isotropic parameter ($U = 0.7$ Å^2) was carried out for ester **2a**. In ester **2e** the hydrogen atoms were included at their calculated positions in the later iterations with unique fixed isotropic $U = 0.7$ Å^2, while in ester **2f** the hydrogen atoms were refined isotropically. The 3 structures converged to R = 0.030 **(2a)**, R = 0.054 (Rw = 0.058) **(2e)**, R = 0.049 (Rw = 0.051) **(2f)** ($R = \Sigma[|Fo|-|Fc|]/\Sigma|Fo|$; $Rw = \{\Sigma w[|Fo|-|Fc|]^2/\Sigma w|Fo|^2\}^{1/2}$). Final atomic parameters and structure factors Fo and Fc have been deposited at the Crystallographic Data Center, Cambridge, England [20].

Neutral atom scattering factors used in all structure-factor calculations were taken from ref. [21]. Data processing and computations were carried out using SHELX 76 program package [19]; PARST program [22] was used for geometrical calculations, and PLUTO [23] for drawings.

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