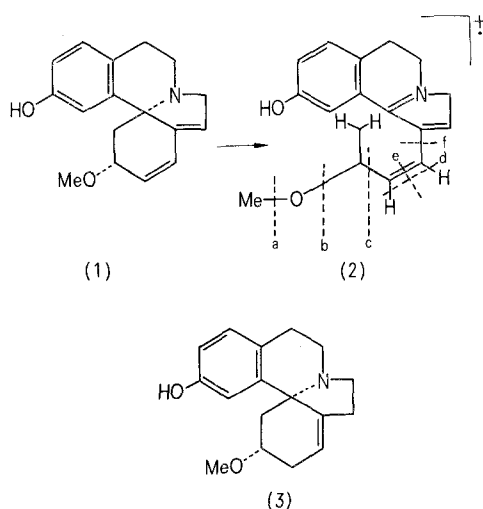


$J_2 = 0.5$  Hz) forming the 'A' part of the ABX system and the third at  $\tau$  4.20. Double resonance experiments<sup>16</sup> enabled us to interpret these data fully. Irradiation at  $\tau$  6.50 ( $\alpha$  to oxygen) caused a collapse of the small (2 Hz) splitting of the olefinic protons at  $\tau$  3.45, leaving the AB system ( $J = 10$  Hz) of the 2 lower field olefinic protons. This infers a *cis* orientation of the double bond. The irradiation also sharpened the signal at  $\tau$  4.02 of the 'A' part of the system indicating 0.5 Hz allylic coupling. These results are accommodated by the 3-methoxy-1,6-diene system of the *Erythrina* alkaloids and imply a 3-4-equatorial conformation for the methoxyl group, as is present in the previously characterised *Erythrina* alkaloids<sup>17, 18</sup>.

The hydroxyl group present in **1** was placed at position C-15 as follows: irradiation at  $\tau$  7.09 (benzylic region) sharpened a low field doublet at  $\tau$  2.92 due to an *ortho* coupled aromatic proton. There was no effect on the other aromatic protons; irradiation  $> 10$  Hz either side of  $\tau$  7.09 had no effect. It follows that the hydroxyl group is at C-15.



The mass fragmentation pattern of the base was in complete agreement with the proposed structure **1**. The prominent ions in the spectrum were  $m/e$  269 ( $M^+$ ); a) 254 ( $M^+ - 15$ ); b) 238 ( $M^+ - 31$  base); c) 211 ( $M^+ - 58$ ); d) 209 ( $M^+ - 60$ ); e) 198 ( $M^+ - 71$ ) and f) 185 ( $M^+ - 84$ ). A rationalisation of this, based on established precedent<sup>19</sup> is given in **2**.

Reduction of coccuvine in methanol with 10% Pd/C afforded dihydrococcuvine which was found identical in all respects with cocculine (**3**)<sup>13</sup> of established stereochemistry; coccuvine must, therefore, have the structure and stereochemistry shown in **1**.

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## Levels of $\alpha_1$ -Antitrypsin in the Spring and Autumn Seasons

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**Summary.** In a group of 84 pairs of 11-year-old children of both sexes, the level of the  $\alpha_1$ -antitrypsin ( $\alpha_1$ -AT) were ascertained in the autumn and spring. Although the mean levels of  $\alpha_1$ -AT in the two seasons hardly differed, the highly significant seasonal changes in the distribution curves of  $\alpha_1$ -AT values were noted in boys, whereas the levels showed higher stability in girls.

The levels of  $\alpha_1$ -antitrypsin ( $\alpha_1$ -AT) in the blood serum are genetically determined (for ref. see <sup>1-3</sup>). On the other hand, the  $\alpha_1$ -AT levels are concomitantly influenced by a number of intrinsic and extrinsic factors: rising in cases of malignant tumors<sup>4</sup>, of different pneumopathies<sup>5</sup>, in pregnancy and parturient women<sup>6</sup>, in macrophages of smokers<sup>7</sup> and in persons injected with typhoid vaccine<sup>8</sup>. The relevance of low levels for the pathogenesis of obstructive pulmonary disease is well known<sup>1-3</sup>. In view of the potential lability of the  $\alpha_1$ -AT levels, we were

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prompted to asses the possible role of seasonal influence.

**Material.** The venous blood was collected from 84 11-year-old children (boys and girls) in October 1974 and in April 1975, living in a town of 8500 inhabitants (Říčany) in Central Bohemia. The blood was taken from fasting children in the course of 4 consecutive days, always between 08.00–09.30 h. After 2 h the blood was centrifuged, the serum harvested and stored at  $-15^{\circ}\text{C}$  until processing, which was done within the following 2 months.

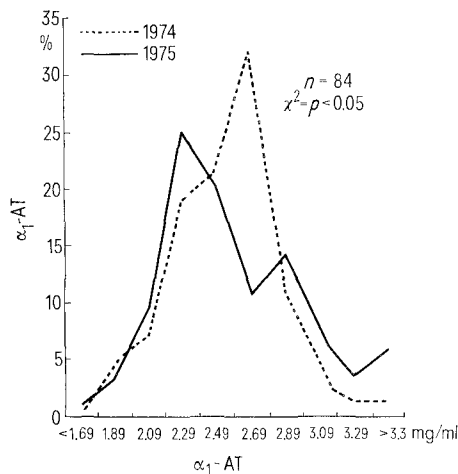


Fig. 1. Distribution of  $\alpha_1$ -AT values in the autumn and spring.

**Methods.** The levels of  $\alpha_1$ -AT were measured by radial immunodiffusion according to MANCINI et al.<sup>9</sup> on plates with the Ionagar Oxoid gel containing monospecific anti-serum against  $\alpha_1$ -AT (Behringwerke Marburg a. d. L.). The reaction of 6 dilutions of the  $\alpha_1$ -AT standard (Protein-Standard-Plasma Behringwerke) was measured on every plate with 36 serum samples. The samples from 1 week were processed in a single day. The error of the method expressed in percentage of the standard deviation from 7 examinations performed with 1 sample was: 1 SD = 3.9%, 2 SD = 7.8%.

The results were evaluated statistically by  $\chi^2$ -test, Kolmogorov-Smirnov test, Student's *t*-test, by assessing regression lines and by the coefficient of correlation.

**Results.** Figure 1 shows the difference in the distribution of the  $\alpha_1$ -AT values in the group observed in the autumn 1974 and in the spring 1975. In the spring sampling, a deformation of the distribution curve is obvious in comparison with the autumn values;  $\chi^2$ -test already showed the significance of the difference of the 2 curves ( $p < 0.01$ ). Another analysis revealed that the difference observed stems almost entirely from a movement of values in the boys' subgroup ( $p < 0.005$ ), whereas in the girls' group the shift was not significant. Figure 2 shows the differences between the individual  $\alpha_1$ -AT values in the 2 seasons. The regression line of  $\alpha_1$ -AT for the boys' group is markedly different compared with that of the girls' group. The correlation of seasonal values in both

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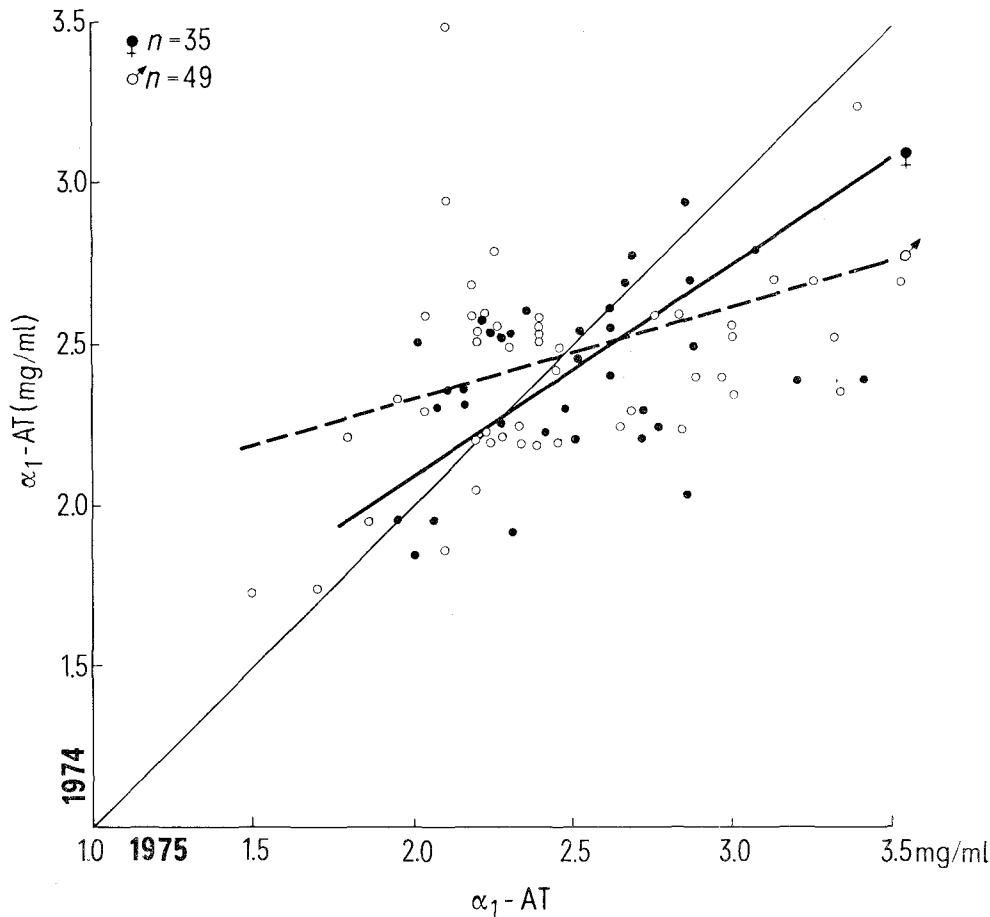


Fig. 2. Scatter of  $\alpha_1$ -AT values 1974 and 1975 and regression lines of boys and girls. Girls:  $r = 0.8616$ , boys:  $r = 0.3568$ .

Mean levels of  $\alpha_1$ -AT in the autumn and spring

Statistics season of the year	Boys				Girls				Boys and girls			
	<i>n</i>	$\bar{x} \pm SD$	Range	95% c.i.	<i>n</i>	$\bar{x} \pm SD$	Range	95% c.i.	<i>n</i>	$\bar{x} \pm SD$	Range	95% c.i.
Autumn 1974	49	2.43 $\pm$ 0.32	1.73–3.5	2.62–2.33	35	2.4 $\pm$ 0.26	1.85–2.95	2.49–2.31	84	2.41 $\pm$ 0.3	1.73–2.5	2.48–2.35
Spring 1975	49	2.47 $\pm$ 0.46	1.5 –3.55	2.6 –2.34	35	2.49 $\pm$ 0.35	1.95–3.4	2.61–2.37	84	2.48 $\pm$ 0.41	1.5 –3.55	2.57–2.39
F-test	$p < 0.05$				Insignificant				$p < 0.01$			
t-test	Insignificant				Insignificant				Insignificant			

sexes is also different:  $r = 0.8616$  between the autumn and spring values in the girls' subgroup is highly significant ( $p < 0.001$ ), whereas in the boys' subgroup  $r = 0.3568$  is only slightly significant ( $p < 0.05$ ).

The Table shows the means of  $\alpha_1$ -AT in boys and girls in the 2 seasons. Except for a significant difference between the value dispersions in boys (F-test  $p < 0.05$ ) and in the whole group (F-test  $p < 0.01$ ) in the 2 seasons, no significant difference of mean levels was found by *t*-test.

There is no correlation between the values of  $\alpha_1$ -AT and proteinemia: in the autumn sampling,  $r = -0.0123$ , in the spring sampling,  $r = -0.0193$ .

*Discussion.* The levels of  $\alpha_1$ -AT could be changed under certain physiological or pathological circumstances, as stated in the introduction. This paper shows the changes of the  $\alpha_1$ -AT values in prepubertal children under seasonal influences. It is interesting that this influence is very strong in boys but not in girls. The  $\alpha_1$ -AT levels in boys in spring tend to shift either to high or to low values. The variability is, therefore, great in both directions. Consequently, the mean values of  $\alpha_1$ -AT in the boys' subpopulation are practically the same in both seasons. However, the differences in the distribution of values are highly significant in the  $\chi^2$ -test, and this result is confirmed by the course of the regression line and by the correlation coefficients. The results suggest a more stable genetic control in girls' subpopulation. It is beyond the scope of this paper to explain why the  $\alpha_1$ -AT levels are more labile in boys seasonally. One reason for this may be a different start of prepubertal hormonal changes in both sexes, while girls are actually physiologically older.

When the  $\alpha_1$ -AT results observed in the autumn sampling are considered as initial values, then the changes in the  $\alpha_1$ -AT levels assessed at the beginning of spring could with some probability be attributed to the influence of the previous winter season. The nutrition factor probably has no significant influence on the formation of  $\alpha_1$ -AT levels, because there is no correlation of  $\alpha_1$ -AT with proteinemia.

However, this season comprises many factors which should be taken into account: low temperature, a reduced vitamin content in fresh fruit and vegetables, increased incidence of infectious diseases and an increased amount of exhalation pollutants in the air. Some of the factors mentioned may be followed in a further study which is now being compiled.

The Mannophosphoinositides of *Nocardia asteroides*

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*Summary.* The phospholipid of *N. asteroides* has been investigated. It was found to contain phosphatidyl ethanolamine, cardiolipin, phosphatidyl inositol and a family of mannophosphoinositides. Dimannophosphoinositides with 3 and 4 moles of fatty acid per phosphate residue represented the major glycopospholipids besides small amounts of other more glycosylated mannophosphoinositides.

The close phylogenetic relationship of *Mycobacteria*, *Corynebacteria* and *Nocardia* is supported by morphological<sup>1</sup> and immunological<sup>2</sup> evidence. Lipids of *Mycobacteria*<sup>3</sup> and *Corynebacteria*<sup>4-6</sup> have been the subject of intensive studies in the recent years whereas little information is available concerning *Nocardia*. *Mycobacteria* contain unusual phosphatidyl myoinositol oligomannoside where the number of mannose units may vary from 1 to 5<sup>7</sup>. However, recent studies suggest that these lipids are more complex and differ in the number of fatty acyl groups<sup>8,9</sup>. The phospholipids of *Nocardia* have been shown to contain cardiolipin, phosphatidyl ethanolamine and either mono or dimannophosphoinositide<sup>10,11</sup>. This report pertains to the nature of mannophosphoinositides of *Nocardia asteroides*.

*Materials and methods.* *Nocardia asteroides* were grown for 4 weeks in a medium containing glucose, beef extract and peptone<sup>11</sup>. Extraction and purification of the lipids

were described elsewhere<sup>12</sup>. Mannophosphoinositides were separated by thin layer chromatography (TLC) using silica gel H plates impregnated with 0.18% ammonium

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