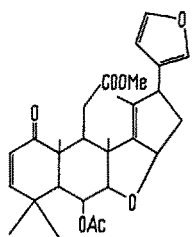
NMR spectra of merolimonol derivatives and quassin[a]

	H_{15}	H_7	H_{19}	H_1	C_{13}	C-methyls at C_8	C_4	Other assignments
Merolimonol (7)	5.09	4.43[e] (<3)	4.29 4.44 (12)	3.98 (3)	1.72 (2)	1.27	1.13 1.05	
Merolimonol acetate (8)	6.09	4.56[d] (<3)	4.32 4.45 (14)	4.01 (3)	1.82	1.27	1.13 1.05	Acetate 2.22
Anhydromerolimonol (9)		4.39[d] (<3)	4.45 4.58 (13)	3.96 (3)	1.87 (1)	1.28	1.18 1.10	H_{12}, H_{14}, 5.57[b] H_{15}, H.., 5.51[c]
Quassin (1)		4.37[d] (<3)				1.22	1.13 (6)	H_3 5.38 (2) H_9, 3.00

[a] The NMR spectra were recorded at 60 mc on a Varian A-60, in deuterochloroform relative to TMS. Coupling constants given in parenthesis c/s. [b] Sharp singlet. [c] Broad, unresolved band. [d] Unsymmetrical; X part of an ABX pattern. [e] Partly obscured by H_{19} resonance.

oxidized to a carboxylic acid group. Decarboxylation can now easily lead to a quassin type A-ring.



Nimbin (10)

This overall biogenetic process would impose certain obvious structural and stereochemical restrictions consistent with structural variations to be found in the limonoids on those simaroubaceous bitter principles of unassigned structure. One could predict that in addition to having the same general stereochemistry as quassin, the C_{15} hydroxy group in glaucarubol (3) should be in the α-configuration[27].

Zusammenfassung. Ein biogenetischer Vorschlag für Simaruba-Bitterstoffe, welcher ihre chemische Struktur, Stereochemie und ihre Entstehung in der Pflanze erklärt, wird vorgelegt.

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[27] *Note added in proof:* J. B-SON BREDENBERG (Chem. and Ind. *1964*, 73) has proposed an identical biogenetic pathway for the simaroubaceous bitter principles. C. R. NARAYANAN et al. (Chem. and Ind. *1964*, 322) has proposed a complete structure for Nimbin[10].

In vitro and *in vivo* Effects of X-Rays and of Iodoacetic Acid on P^{32} Incorporation into Phosphoethanolamine of Rat Thymus Cells

Orthophosphate labelled with P^{32} has been very largely employed in the study of the effects of ionizing radiation on nucleic acid synthesis and on turnover of nucleotides related to that synthesis, in many living systems[1,2]. Only a few authors so far have studied, with the same precursor, the radiation effects on the turnover of phosphorylated non-nucleotide intermediates in nature[3].

The incorporation of P^{32}-orthophosphate into these intermediates has been examined in previous experiments carried out in this laboratory *in vitro* with isolated rat thymus cells[4]. These experiments, dealing with the study of the mechanism of radiosensitizing effect of iodoacetic acid (IAA), showed that both X-rays and IAA induced an inhibition of P^{32}-incorporation only on a single component, which was identified as phosphoethanolamine (PEA).

In the present paper the results of a more detailed study of the effects of X-rays and of IAA on the incorporation of P^{32}-orthophosphate in rat thymocytes *in vivo* and *in vitro* are reported.

Carrier free P^{32}-orthophosphate, from Radiochemical Centre, Amersham, was diluted with inert KH_2PO_4-

[1] Z. M. BACQ and P. ALEXANDER, *Fundamentals of Radiobiology* (Pergamon Press, 1961).

[2] M. G. ORD and L. A. STOCKEN, in M. ERRERA and F. FORSSBERG, *Mechanisms in Radiobiology*, (Academic Press, 1961), vol. 1.

[3] F. BRESCIANI and K. DOSE, Radiobiologia, Radioterapia e Fisica Medica *15*, 313 (1959).

[4] P. MISITI DORELLO, M. BOCCACCI, M. QUINTILIANI, and E. STROM, Rend. Ist. Sup. Sanità, in stampa.

solution so that a final concentration of 100 μg/ml of P with an activity of 500 μc/ml was obtained. Female Wistar Glaxo rats, bred in this Institute, weighing 80–120 g were employed. Methods for preparation, irradiation and incubation with P^{32} of thymus cell suspensions were the same as reported in the previous paper[4]. The pooled contents of two Warburg vessels, containing suspensions treated in the same way, were analysed.

For *in vivo* experiments the animals were given intramuscularly 0.1 ml of diluted P^{32}-solution (50 μc), killed with ether 2 h later and the thymus was quickly removed and homogenized in chilled perchloric acid. Two thymi were pooled and homogenized in 19 ml of 0.5 *M* perchloric acid. X-irradiation was administered by means of a Siemens therapy machine working at 220 kV, 15 mA with an added filtration of 0.5 mm Cu and 1 mm Al. The animals – kept in groups of 4 in a wooden container – were irradiated at a dose rate of 110 r/min.

Phosphorylated non-nucleotide compounds, extracted and purified as previously reported[4], were separated by chromatography on Dowex 50 × 8 resin, 200–400 mesh, in column 1 × 50 cm, with citrate buffer pH 2.53[5]. On each eluted fraction, the radioactivity was measured and the ninhydrin reaction performed[6]. The identification of PEA was achieved as previously reported[4].

The chromatographic pattern obtained with purified extracts shows two radioactive peaks, only the second of which is ninhydrin positive. All phosphorylated intermediates not containing basic functions are contained in the first peak. In the second peak a radioactive ninhydrine positive component was found. This component has been identified as PEA, and this is the only component of this fraction labelled with P^{32} and reacting with ninhydrine.

The results of *in vitro* experiments are reported in Table I. The data show that X-rays as well as IAA significantly inhibit the incorporation of P^{32} into PEA, whereas the amount of this substance in the aliquot of the cell extract employed for the analysis remains unchanged. With both treatments the inhibitory effect appears to be dose dependent and for X-rays this dependence is of exponential type.

Table II shows the results of *in vivo* experiments. A whole-body irradiation with 1000 r induces a significant inhibition of P^{32}-incorporation into thymus PEA. The inhibition increases with the time up to 8 h after the irradiation. Also *in vivo*, the amount of PEA present in the aliquot of the analysed tissue extract is not altered.

The present observations are not sufficiently extended to allow a discussion of the biological significance of the radiation and IAA effects on the incorporation of phosphate into PEA. We can only recall that PEA is considered to be an intermediate product in the phospholipid metabolism[7]. In this light these effects could be of some interest in relation to the hypothesis suggesting that the mechanism of action of ionizing radiation in living systems may be primarily concerned with alterations of the permeability of cellular and intracellular membranes[8].

According to recent authors, the active transport phenomena at the level of these membranes would be intimately connected with the metabolism of phosphatidic acids present in the lipid fraction of the membranes[9]. Phosphoethanolamine can be considered a precursor of the phosphatidylethanolamine.

In this connection, it can be pointed out that a lower uptake of P^{32} into the phospholipid fractions, containing PEA, of the liver and spleen of irradiated mice has recently been reported[10,11].

Table I. Effects of iodoacetic acid and of X-rays on the incorporation of P^{32} into phosphoethanolamine of rat thymus cells *in vitro*

	Specific activity c.p.m./μg PEA	PEA μg[a]
Control	100 ± 4	3.7 ± 1.2
Iodoacetic acid 10^{-4} *M*	68 ± 4 ($P = 0.01$)	3.6 ± 1.0
Iodoacetic acid 5×10^{-4} *M*	40 ± 3	3.6 ± 1.1
X-rays 720 r	50 ± 3 ($P = 0.01$)	3.5 ± 1.0
X-rays 1 440 r	25 ± 1	3.7 ± 1.2
X-rays 2 160 r	15 ± 2	3.6 ± 0.9

[a] Amount present in the fraction on which the radioactivity was determined.

Table II. Incorporation of P^{32} into phosphoethanolamine of rat thymus at various times after irradiation with 1000 r of X-rays. Rats were injected intramuscularly with 50 μc of P^{32} 2 h before death. Times reported in the Table correspond to 1 h after P^{32} administration

	Specific activity c.p.m./μg PEA	PEA μg[a]
Control	100 ± 5	5.5 ± 1.1
1 h after irradiation	75 ± 7 ($P = 0.05$)	5.4 ± 1.2
5 h after irradiation	55 ± 4	5.6 ± 1.0
8 h after irradiation	25 ± 3	5.5 ± 1.3

[a] Amount present in the fraction on which the radioactivity was determined.

Riassunto. Sono stati studiati *in vivo* ed *in vitro* gli effetti dei raggi e dell'acido iodoacetico sulla incorporazione di P^{32} nella fosfoetanolamina «acido solubile» del timo di ratto.

È stato osservato che ambedue i trattamenti determinano *in vitro* una marcata inibizione della incorporazione nella fosfoetanolamina del P^{32}, somministrato come ortofosfato, e che l'entità della inibizione è proporzionale alla dose.

Per i raggi è stato trovato *in vivo* che l'entità della inibizione aumenta progressivamente dal momento dell'irraggiamento sino a raggiungere valori del 75% dopo 8 h.

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[11] *Acknowledgment.* The authors are indebted to Mr. L. BERNARDINI for technical assistance.