with a similar result. The treated plants produced their first flower buds 12 days after the controls.

A second set of experiments was devised to determine whether or not indole acetic acid solutions might inhibit the development of already-formed flower buds into fully opened flowers. Young inflorescences bearing flower buds in various stages of development but no open flowers were cut from plants of stocks, snapdragons (Rose Queen), annual larkspur (Blue Bell), blue salvia, and iris (Sierra Blue), and were immediately placed with their cut ends in water solutions of indole acetic acid (25, 50, 100, 150 ppm) for 24 hrs; following this treatment, the inflorescences were placed in containers with the cut ends in tap water. On successive days, counts were made of the numbers of buds which developed into fully opened flowers. The results indicate that, in all treated plants, the indole acetic treatment retarded the development of flower buds into open flowers, as compared with that of untreated controls. In iris, the growth of flower buds was completely inhibited; all buds of the controls opened. The retarding effect of the auxin solutions upon bud development was more pronounced at the higher concentrations used; also, at the higher concentrations, some signs of leaf epinasty and of abnormal stem twisting were observed. These growth abnormalties were less pronounced than those of leaves directly treated with auxin sprays.

Although these experiments differed somewhat from those of Thurlow and Bonner, the results are similar to theirs. It cannot be assumed that the results of such experiments demonstrate a causal relationship between auxin content of a plant and its flowering, but these preliminary tests may open a way to more precise approaches to the suggested relationship.

References

1. CHOLODNY, N. G. *Herb. Rev.*, 1940, **7**, 223–247.
2. THURLOW, JOHN, and BONNER, JAMES. *Amer. J. Bot.*, 1947, **34**, 25a–26a. (Abstract.)

Observation on the Mechanism of Action of Dicoumarol

ROBERT L. MACMILLAN

Department of Medicine, University of Toronto

The mechanism of the action of dicoumarol is obscure, and many clinicians hesitate to use it for this reason. Quick (*5*) presents evidence that the prothrombin time, as measured by the one-stage method, may be prolonged by diminution of prothrombin component A, component B, or a labile factor. According to Quick, component B is reduced in dicoumarol administration.

Loomis and Seegers (*2*) consider prothrombin to be a unitary principle and receive support from Munro and Munro (*3*), who have demonstrated that A and B cannot be regarded as separate components.

Accepting the hypothesis of a unitary principle, the clotting time has been studied when dicoumarol plasma is mixed with whole normal plasma, Seitz-filtered plasma, defibrinated plasma, and serum.

Experiment 1—Mixture of Dicoumarol Plasma and Normal Plasma

Dicoumarol plasma (14% prothrombin)		Dilution of normal plasma				
Mixture		80%	50%	25%	12.5%	6.25%
Prothrombin percentage	Observed %	50	42	38	34	22
	Arithmetic avg. %	47	32	19	13	10

Experiment 2—Dicoumarol Plasma and Seitz-filtered Normal Plasma

Dicoumarol plasma (5% prothrombin)		Dilution of Seitz-filtered plasma (free from prothrombin)				
Mixture		80%	50%	25%	12.5%	6.25%
Prothrombin percentage	Observed %	3	3	3	2	3
	Arithmetic avg. %	3	3	3	3	3

Experiment 3—Dicoumarol Plasma and Defibrinated Normal Plasma

Dicoumarol plasma (25% prothrombin)		Dilution of defibrinated plasma* (100% prothrombin)			
Mixture		50%	25%	12.5%	6.25%
Prothrombin percentage	Observed %	30	24	20	17
	Arithmetic avg. %	37	25	18	15

* Plasma was defibrinated by the addition of 1/10 volume of Parke Davis & Co. Thrombin Topical: One ampoule was made up to 200 ml with 0.15 N sodium chloride. The excess thrombin was inactivated by keeping the plasma at 37° C for 1 hr.

Experiment 4—Dicoumarol Plasma and Normal Serum

Dicoumarol plasma (24% prothrombin)		Dilution of 24-hr serum* (trace of prothrombin)			
Mixture		50%	25%	12.5%	6.25%
Prothrombin percentage	Observed %	44	38	32	24
	Arithmetic avg. %	12	12	12	12

* The serum was allowed to stand for 24 hrs. It contained a trace of prothrombin by the one-stage method, but no thrombin.

Dilutions of normal were made with 0.9% sodium chloride; 0.1 ml of dicoumarol plasma and 0.1 ml of diluted normal plasma were added to 0.2 ml of thromboplastin (Difco) and the mixture activated by 0.2 ml of 0.025 M calcium chloride solution. The clotting time was measured at 37° C and the corresponding prothrombin percentage read from a dilution curve. This value was compared with the arithmetic average of the prothrombin concentrations of the dicoumarol plasma and the normal plasma, measured separately.

The recorded observations are ones selected at random from experiments using several different dicoumarol plasmas.

When normal plasma is added to dicoumarol plasma, the prothrombin content of the mixture is increased more than can be explained by the simple mixing of prothrombin solutions of different concentrations. Conley and Morse (*1*) recognized this when they added incoagulable plasma from a dog receiving large quantities of dicoumarol to normal human plasma.

This observation could be explained by the presence of a prothrombin accelerator in normal plasma which is deficient in dicoumarol plasma. When observed values for the mixture are plotted against arithmetically expected values, a logarithmic curve is obtained.

The accelerator is removed from the plasma by Seitz filtration. This distinguishes it from Owren's (*4*) factor V, which passes through Seitz filters.

The accelerator disappears when normal plasma is treated with thrombin. It is present, however, in serum.

References

1. Conley, C. L., and Morse, W. I. *Amer. J. med. Sci.*, 1948, **215**, 158.
2. Loomis, E. C., and Seegers, W. H. *Amer. J. Physiol.*, 1947, **148**, 563.
3. Munro, M. P., and Munro, F. L. *Amer. J. Physiol.*, 1947, **150**, 409.
4. Owren, P. A. *Acta Med. Scand.* (Suppl. 194 on the Coagulation of the Blood), 1947.
5. Quick, A. J. *Amer. J. Physiol.*, 1947, **151**, 63.

Isolation of Pure Vitamin A_2[1]

Edgar M. Shantz

Research Laboratories, Distillation Products, Inc., Rochester, New York

Since the discovery in 1937 of vitamin A_2 (*1*, *4*), a compound related to vitamin A and found chiefly in the livers of certain species of fresh-water fish, there has been much disagreement among various investigators with respect to its structure and biological activity. Much of this dissension arises from the lack of criteria by which to measure vitamin A_2. The purpose of this paper is to establish some of these criteria by reporting the isolation of pure (though noncrystalline) vitamin A_2 alcohol through a crystalline ester.

During the fall of 1946, 150 lbs of "pike" livers were collected from local fish markets. Because the origin of the livers could not be definitely assured, each liver was examined under an ultraviolet lamp. Livers showing the brilliant yellow fluorescence of vitamin A_1 were discarded, while those showing the characteristic brownish-orange fluorescence of vitamin A_2 were pooled, ground, and extracted with ethyl ether. Slightly over 4 kg of rather low-potency oil was obtained.

In the following brief description of the concentration of vitamin A_2 from this oil, the symbol E stands for E (1%, 1 cm) at 351 mμ, the main ultraviolet absorption maximum of vitamin A_2.

The original oil (E = 4.0) was distilled in a centrifugal molecular still to give a distillate containing the natural vitamin A_2 esters (E = 24.2). Redistillation further increased the potency (E = 65.0), and the triglycerides were removed by saponification (E = 182). Sterols were crystallized out in acetone at – 30° C (E = 260) and the residue chromatographed on sodium aluminum silicate (E = 482). The chromatographed concentrate was distilled in a high-vacuum pot still (E = 848) and desterolated again in ethyl formate at – 30° C (E = 862). The concentrate was then chromatographed on magnesium oxide (E = 1,030), on zinc carbonate (E = 1,250), again on zinc carbonate (E = 1,320), and a third time on zinc carbonate (E = 1,350). The extinction could not be increased by further chromatography. This is about the same extinction as that obtained by Karrer and Bretscher (*3*) in their investigations of vitamin A_2. However, other data reported by these workers (main ultraviolet absorption maximum at 345 mμ instead of 351 mμ, along with relatively low extinctions for the subsidiary band at 287 mμ and for the $SbCl_3$ blue product at 695 mμ) indicate that their preparation probably contained appreciable quantities of vitamin A_1.

Since attempts to crystallize this material from various solvents (methyl alcohol, methyl formate, ethyl formate, Skellysolve "F") were unsuccessful, 1.25 gm of the concentrate was esterified with 1.4 gm of phenylazobenzoyl chloride by allowing it to stand for 4 hrs at room temperature in 20 ml of methylene chloride containing 2 ml of pyridine.

The vitamin A_2 phenylazobenzoate thus obtained was chromatographed twice on zinc carbonate. The ester formed a weakly adsorbed reddish-orange band which was eluted from the column with petroleum ether (Skellysolve "F"). The ester concentrate (1.9 gm) was allowed to stand in 10 ml of Skellysolve "F" at – 30° C. After 3 days, crystals formed in hemispherical orange rosettes on the walls of the vessel. Three recrystallizations gave a final yield of 329 mg of tiny orange prisms which melted at 76°–77° C (Fisher-Johns apparatus, uncorr.). *Analysis*: required for $C_{33}H_{38}N_2O_2$ (vitamin A_2 phenylazobenzoate using Karrer's open-chain formula)—C, 80.12; H, 7.74; N, 5.66; *Found*: C, 80.1; H, 7.8; N, 5.9. The compound had an ultraviolet absorption maximum at 341 mμ with E (1%, 1 cm) = 1,190. This shift to a lower wave length is due to the acid moiety, which has its own strong absorption band at 330 mμ.

Careful saponification under nitrogen of 258 mg of crystals gave 152 mg (theory, 150 mg) of vitamin A_2 alcohol. Adsorption on a small column of zinc carbonate showed a single homogeneous band with the exception of some strongly adsorbed red material (2 mg) at the top of the column and a tiny, weakly adsorbed orange band (about 1 mg) at the bottom of the column. These impurities were removed, and the vitamin A_2 eluted from the rest of the column was considered to be pure.

This preparation was a viscous, orange-yellow oil which showed two absorption maxima in the ultraviolet, one

[1] Communication No. 133 from the Laboratories of Distillation Products, Inc., Rochester, New York.