

acetate obtained by reductive acetylation of the pigment.

For preparation of the acetate, a suspension of 8 mg of the *Phlebia* pigment in 2 ml of acetic anhydride was kept at room temperature overnight. The solution was dried under reduced pressure and the residue was recrystallized from methanol, giving a high yield of yellow needles melting at 233° to 234°C. Recrystallization from ethyl acetate or from cyclohexane yielded yellow needles melting at 190° to 191°C. The IR spectra of the two forms in CHCl₃ solution of KBr disc were indistinguishable. The ultraviolet absorption spectra had $\lambda_{\text{max}}^{\text{EtOH}}$ 262 m μ (log ϵ , 4.39) and a shoulder, 310 to 340 m μ (log ϵ , about 3). The melting point of a mixture of the two forms lay between the respective melting points. For analysis, a sample of the higher-melting form was dried under reduced pressure at about 117°C. Found: C, 56.29; H, 4.04; O, 39.97. Calculated for C₃₀H₁₈O₁₂: C, 55.70; H, 3.80; O, 40.48.

Both forms of the acetate could be obtained also from authentic oosporein (from *V. psalliotae*), and these samples did not depress the melting point of samples of the *Phlebia* pigment tetraacetate; IR spectra were identical (Fig. 1).

The leucoacetate was prepared by shaking a suspension of 10 mg of *Phlebia* pigment and 10 mg of PtO₂ catalyst in 10 ml of acetic anhydride in a hydrogen atmosphere; after 30 minutes the mixture became colorless. The catalyst was filtered off and the solvent was removed under reduced pressure. Recrystallization of the residue from methanol yielded 10 mg of colorless prisms melting at 250° to 251°C. These were dried under reduced pressure at about 117°C for analysis. Found: C, 55.97; H, 4.66; O, 38.39; molecular weight, 582. Calculated for C₃₀H₃₀O₁₆: C, 55.72; H, 4.68; O, 39.60; molecular weight, 646.

The melting point of the leucoacetate was not depressed by mixture with a sample of the corresponding derivative prepared from authentic oosporein and their IR spectra were identical (Fig. 1). The ultraviolet absorption spectra had $\lambda_{\text{max}}^{\text{EtOH}}$ 272 m μ (log ϵ , 3.43). The butyrate was prepared by warming a solution of 15 mg of the pigment in 5 ml of butyric anhydride on the steam bath for 30 minutes. The yellow crystalline residue left by evaporation of the solvent was recrystallized from cyclohexane and yielded 8 mg of yellow prisms melting at 105° to 107°C. Found: C, 61.42; H, 5.84; O, 32.74.

Calculated for C₃₀H₃₄O₁₂: C, 61.47; H, 5.84; O, 32.83.

Identity of *P. albida* pigment with *P. mellea* pigment was established by comparison of the leucoacetates. The crude pigment obtained from *P. albida* was acetylated directly with acetic anhydride; recrystallized from methanol, the product yielded yellow crystals. The IR spectra of these indicated a mixture of oosporein acetate and leucoacetate. Reductive acetylation of this mixture with acetic anhydride and zinc dust on the steam bath for 30 minutes yielded a crystalline solid, which was purified by adsorption on a silica gel column and elution with a mixture of benzene and ethyl acetate. The IR spectrum of this leucoacetate (KBr pellet) was identical with that of the leucoacetate from *P. mellea*.

The nmr spectrum of the *Phlebia* pigment in deuterodimethylsulfoxide showed only a single peak at τ 8.13 (aromatic methyl protons); no aromatic hydrogen was observed. Hydroxyl proton peaks were obscured by background, but the acetate showed two sets of acetoxymethyl protons at τ 7.65 and τ 7.75, besides the aromatic methyl protons, τ 7.97 (relative intensities 6:6:6). The leucoacetate showed three separate methyl peaks: τ 7.73 and τ 7.95 acetoxymethyl and τ 7.93 aromatic methyl (relative intensities 12:12:6). Neither acetate showed a peak in the aromatic proton region.

Vining, Kelleher, and Schwarting (9) likewise identified as oosporein a red pigment produced by a culture originally identified as a strain of the basidiomycete *Amanita muscaria*; they were investigating this strain as a possible good producer of the terphenyl quinone pigment, muscarufin. But the culture, like another supposed basidiomycete culture cited, proved to be a *Beauveria* (Fungi Imperfecti).

Since Vining *et al.* made it apparent that mistaking a species of *Beauveria* for a basidiomycete is quite possible, we felt that thorough checking of our *Phlebia* cultures was called for. Rogerson, of this institution, examined our cultures of *P. mellea* and *P. albida* and reported that the mycelia are septate and produce clamp connections; no evidence of the presence of Fungi Imperfecti could be found.

To our knowledge, the only other naturally occurring bibenzoquinone reported is phoenicin (12), isolated from a *Penicillium* (13). This is therefore the first reported isolation of a compound of this type from a basidiomycete. Although leucophoenicin is reported to

occur (14) we have found no previous report of the natural occurrence of the leuco form of oosporein. Its presence supports the suggestion (6) that oosporein, like phoenicin (13), may function as a respiratory pigment.

HITOSHI TAKESHITA

MARJORIE ANCHEL

New York Botanical Garden,
Bronx Park, Bronx 10458

References and Notes

1. The *Phlebia mellea* was culture No. 16642 obtained from Dr. M. K. Nobles, Department of Agriculture, Central Experimental Farm, Ottawa, Canada; the *Phlebia albida* culture No. 21327, from Dr. H. S. Jackson, University of Toronto. Pigment production was also observed in other strains of these species.
2. T. C. McMorris and M. Anchel, *Tetrahedron Letters* No. 5, 335 (1963).
3. Analyses performed by Dr. F. Pascher, Bonn, Germany.
4. Nuclear magnetic resonance spectra were done by Varian Associates and Nuclear Magnetic Resonance Specialties.
5. R. Kuhn and H. Roth, *Chem. Ber.* **66**, 1274 (1933); F. Pregl, *Quantitative Organic Microanalysis* (Churchill, London, ed. 5, 1951), p. 206.
6. F. Kögl and G. C. van Wessem, *Rec. Trav. Chim.* **63**, 5 (1944).
7. G. Lloyd, A. Robertson, G. B. Sankey, W. B. Whalley, *J. Chem. Soc.* **1955**, 2163 (1955).
8. P. V. Divekar, R. H. Haskins, L. C. Vining, *Can. J. Chem.* **37**, 2097 (1959).
9. L. C. Vining, W. J. Kelleher, A. E. Schwarting, *Can. J. Microbiol.* **8**, 931 (1962).
10. J. Smith and R. H. Thomson, *Tetrahedron* **10**, 148 (1960), ref. 5 to H. W. Reading, thesis, London Univ. (1955).
11. Cooked potato (200 g) plus dextrose (20 g) per liter of medium.
12. T. Posternak, *Helv. Chim. Acta* **21**, 1326 (1938).
13. E. A. H. Friedheim, *ibid.*, p. 1464.
14. T. Posternak, *Compt. Rend. Soc. Phys. Hist. Nat. Genève* **56**, 28 (1939).
15. Supported by grant E226 from the National Institutes of Allergy and Infectious Diseases. We thank H. Shaw and S. Shreni for technical assistance.

19 October 1964

Pyramidal Tract: A Comparison of Two Prosimian Primates

Abstract. *The pyramidal tract of the slow loris (Nycticebus coucang) is found in the lateral funiculus of the spinal cord and extends throughout its entire length. Such a course is typical of primates. In the Malayan tree shrew (Tupaia glis) the tract occupies a position in the ventral portion of the dorsal funiculus, and in our studies it could not be traced beyond the thoracic cord. In the spinal cord of the slow loris, pyramidal fibers are distributed to the dorsal, intermediate, and ventral gray columns of both sides, while in the tree shrew they are largely restricted to the dorsal horn and do not cross to the opposite side.*

Considerable information may be found in the literature concerning the course of the pyramidal tracts in various animals. At present some data are

available for representatives of almost all the extant orders of mammals. The pyramidal tract is found in the dorsal funiculus in monotremes, edentates, marsupials, and rodents. A ventral pyramidal tract has been described in elephants and insectivores, while a lateral tract has been found in ungulates, carnivores, lagomorphs, and primates (1). Thus, although the position of the tract is variable from order to order, no instance of variation in position of the major crossed pyramidal tract has yet been described within the same order. Because of this constancy, the finding of a lateral pyramidal tract in the lagomorphs was used as additional evidence that they should be removed from the Order Rodentia (2). Nevertheless, an occasional species within an order has been found to possess, in addition to the major crossed tracts, small, short tracts in one or both other funiculi.

In the primates the major portion of the pyramidal fibers undergo decussation in the caudal medulla to form the crossed lateral pyramidal (corticospinal) tract. In man, chimpanzee, and rhesus monkey a small, short ventral tract is also present. The pyramidal system has not previously been examined in any prosimian primate. Woollard (3) describes a transverse Weigert-Pal section through the pyramidal decussation of *Tarsius* in which the decussating fibers appear to pass to the lateral funiculus, but no comment is made concerning the course of the tract. In view of the lack of information on the anatomy of the pyramidal system in the more primitive primates we have undertaken the study of the slow loris (*Nycticebus coucang*), a lorisiform lemur, and the Malayan tree shrew (*Tupaia glis*). These two species represent crucial stages in a graded phylogenetic series from insectivores through nonhuman primates to man (4).

Twelve specimens of *Tupaia* and eleven specimens of *Nycticebus* were examined. Lesions were made in both species by subpial aspiration in the left cerebral hemisphere. The lesions were so placed as to include various cortical areas including the sensory-motor cortex. After a suitable period the animals were killed by perfusion with 10 percent formol-saline. The brains and spinal cords were then removed and prepared by means of the Nauta-Gygax technique for degenerating

axons (5). Seven of the tree shrews and five of the slow lorises showed massive degeneration of the left medullary pyramid and the right pyramidal tract. The remaining animals had lesions in cortical areas which apparently do not contribute fibers to the pyramidal system. The findings in these animals are not discussed here.

In *Nycticebus* (Fig. 1A) the degenerating pyramidal fibers cross at the decussation and occupy a position in the contralateral lateral funiculus of the cord. The decussation is complete and the tract traverses the entire length of the cord. Contralateral preterminal degeneration was found in the lateral tegmentum of the medulla, and in the cuneate and gracile nuclei. In the gray matter of the cord preterminal degenerating fibers were found to be most numerous in the base of the dorsal horn and the intermediate gray column. Some degenerating fibers were also present in the ventral horn. Degenerating fibers from the contralateral corticospinal tract could be seen to pass through the dorsal and ventral gray commissures to the ipsilateral dorsal, intermediate, and ventral gray columns.

In *Tupaia* (Fig. 1B) the decussation

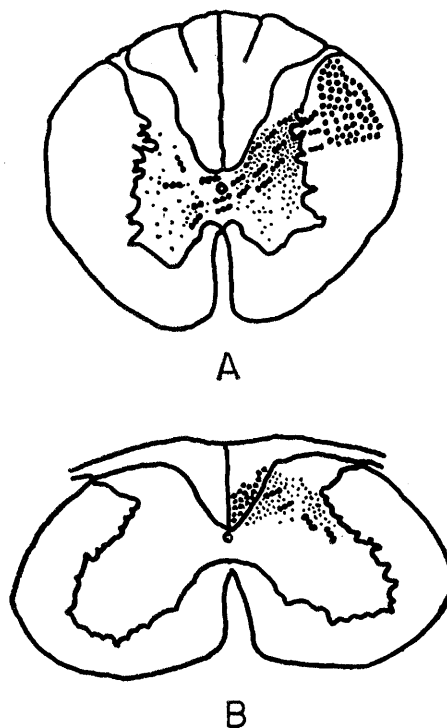


Fig. 1. Degeneration of fibers in the cervical cord of *Nycticebus coucang* (A), and *Tupaia glis* (B). Coarse dots indicate fibers of passage; fine stipple, preterminal degeneration.

in the medulla does not appear to be complete, for in some of our specimens a small bundle of degenerating fibers was seen passing to the ipsilateral corticospinal tract. Contrary to the findings in every primate examined heretofore, the corticospinal tract of the tree shrew is found in the dorsal funiculus. The decussating fibers begin to form the tract just lateral to the nucleus cuneatus. As the tract progresses down the cord it takes up a position in the ventral portion of the dorsal white column.

The number of degenerating fibers was much reduced at the mid-thoracic level and no degenerating fibers were found in the dorsal funiculus of the lumbo-sacral cord. A small number of apparently degenerating fibers were found in the ipsilateral dorsal funiculus in the cervical and thoracic regions of the spinal cord. Contralateral preterminal degeneration in the cord was confined almost entirely to the dorsal gray column. Only isolated fibers could be traced to the zona intermedia and none appeared to extend into the ventral gray column. The heaviest concentration of degenerating fibers occupied the medial aspect of the dorsal horn. No degenerating fibers were found crossing through the gray commissures to the opposite side.

Thus, in the material now available to us there is not only a striking contrast between the tree shrew and slow loris in the position of the tract within the white matter of the cord, but apparently there is a significant difference in the mode of termination of the pyramidal fibers in the spinal gray matter. In *Nycticebus* there appears to be a more direct cortical projection upon the lower motor neurons in contrast to the situation in *Tupaia*. Presumably this offers the expanding neocortex more direct control over motor function. Kuypers (6) has described a similar tendency in the higher primates as compared to the cat.

The localization of the corticospinal tract of the tree shrew in the dorsal funiculus of the spinal cord may have some bearing on the controversy concerning the systematic relationships of the Tupaiidae. These animals were first classified within the Order Insectivora. That the tupaiids are more closely related to the primates was first suggested by Carlsson (7). LeGros Clark (8) has adduced much evidence in favor of this notion. In 1931 Simpson (9) described *Anagale*, supposedly a fossil

tupaoid, which he thought demonstrated significant lemuroid characteristics. Since that time *Anagale* has occupied a prominent place in the arguments supporting a primate status for the tree shrews. Simpson (10), on the basis of the lemuroid tendencies of *Anagale* and the work of LeGros Clark, classified the tree shrews as the superfamily Tupaioidea and placed them with the lemuriform lemurs in the same infraorder.

This classification has been accepted by many comparative anatomists and by most primatologists and physical anthropologists. Some workers who have dissented include: Evans (11), Roux (12), Haines (13), Straus (14), and Osman Hill (15). In addition, McKenna (16) has recently shown that *Anagale* is neither a primate nor a tupaoid. Usually, the numerous non-primate characteristics of the tree shrews have been dismissed by attributing them to their primitive insectivore ancestry. In other words they are "characters of common inheritance." This does not appear to be a likely explanation so far as the localization of the pyramidal tract is concerned. In the insectivores examined thus far—the European hedgehog and two species of mole—uncrossed ventral tracts have been reported (17). Thus, crossed dorsal pyramidal tracts do not appear to be an insectivore characteristic. Straus (14) has suggested that the tree shrews should more properly be placed in a separate order, and our experiments may be interpreted as supporting that conclusion. The present finding does not resolve the controversy, but when considered with other morphological evidence (11–13) it is suggestive that the tree shrews indeed may not be primates.

J. A. JANE*

C. B. G. CAMPBELL†

D. YASHON

Departments of Neurological Surgery and Anatomy, University of Illinois at the Medical Center, and Lincoln Park Zoological Gardens, Chicago, Illinois

References and Notes

1. C. Ariens Kappers, G. Huber, E. Crosby, *The Comparative Anatomy of the Nervous System of Vertebrates, Including Man* (Macmillan, New York, 1936), pp. 271–274; A. Lassek, *The Pyramidal Tract* (Thomas, Springfield, 1954), pp. 62–63; W. Verhaart, *World Neurol.* 3, 43 (1962).
2. A. Douglas and M. Barr, *Rev. Can. Biol.* 9, 118 (1950).
3. H. H. Woollard, *J. Anat.* 62, 276 (1928).

4. W. E. LeGros Clark, *The Antecedents of Man* (Quadrangle, Chicago, 1960), p. 46.
5. W. J. H. Nauta and P. Gyax, *Stain Technol.* 29, 91 (1954).
6. H. Kuypers, in *Structure and Function of the Cerebral Cortex*, D. Tower and J. Schade, Eds. (Elsevier, Amsterdam, 1960), p. 138.
7. A. Carlsson, *Acta Zool.* 3, 227 (1922).
8. W. E. LeGros Clark, *Proc. Zool. Soc. London* 1925, 1185 (1925); *ibid.* 1926, 1179 (1926); *ibid.* 1933, 975 (1933).
9. G. G. Simpson, *Amer. Mus. Novitates* No. 505, p. 1 (1931).
10. G. G. Simpson, *Bull. Amer. Mus. Natur. Hist.* 85, 1 (1945).
11. F. G. Evans, *ibid.* 80, 85 (1942).
12. G. Roux, *Acta Zool.* 28, 165 (1947).
13. R. Haines, *Proc. Zool. Soc. London* 125, 761 (1955); *Quart. Rev. Biol.* 33, 1 (1958).
14. W. Straus, *Quart. Rev. Biol.* 24, 200 (1949).
15. W. C. Osman Hill, *Primates: Comparative Anatomy and Taxonomy. I. Strepsirhini* (Interscience, New York, 1953), p. 5.
16. M. McKenna, *Amer. Mus. Novitates* No. 2158, p. 1 (1963).
17. E. Bischoff, *Anat. Anz.* 18, 348 (1900); J. Dräseke, *Monatsschr. Psychiat. Neurol.* 15, 401 (1904); A. Linowiecki, *J. Comp. Neurol.* 24, 509 (1914).
18. Supported by a grant-in-aid 37074 from Presbyterian-St. Luke's Hospital, Chicago.
- * Present address: Department of Neurosurgery, Western Reserve University Hospitals, Cleveland, Ohio.
- † Present address: Department of Neurophysiology, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C.

3 December 1964

Gibberellin Production in Pea Seeds Developing in Excised Pods: Effect of Growth Retardant AMO-1618

Abstract. *The gibberellin content of pea seeds developing in excised pea pods cultured on a medium containing the plant growth retardant AMO-1618 was reduced in comparison with pea seeds cultured on retardant-free medium. The reduction increased with increasing concentrations of AMO-1618. However, at the lowest concentration tested (5 milligrams per liter) the growth of the seeds was not affected whereas their gibberellin content was significantly reduced. In conjunction with earlier work on the effect of growth retardants on gibberellin biosynthesis in the fungus Fusarium moniliforme, these results indicate that AMO-1618 inhibits the biosynthesis of gibberellins in tissues of higher plants in much the same manner it does in Fusarium.*

The plant growth retardants are a group of chemically unrelated compounds which have the property of reducing the growth of plants. They affect mainly the growth of the stem (internodes); other growth processes and the over-all growth pattern of the plant remain essentially unaffected. The effect of the retardants on stem growth can in most cases be overcome by the application of gibberellin (see 1).

In previous communications it has been shown that the retardants AMO-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride) and CCC ([2-chloroethyl]-trimethylammonium chloride) suppress gibberellin production in cultures of the fungus *Fusarium moniliforme* (*Gibberella fujikuroi*), the first organism in which gibberellin was discovered, without affecting its growth (2). This suppression was found to be due to the inhibition of gibberellin biosynthesis, and not to destruction or inactivation of gibberellin already produced, or other causes (3). There is excellent agreement between the growth-retarding effect of a series of CCC analogs in higher plants and their activity in inhibiting gibberellin biosynthesis in *Fusarium* (4).

In this report we provide direct evidence that the production of gibberellin in higher plants is inhibited by AMO-1618; this inhibition is very probably the physiological basis for the growth effects of this and, presumably, certain other plant growth retardants.

The experiments were conducted with young fruits (pods) of peas (*Pisum sativum* L., cv. Progress No. 9, a dwarf variety) detached from the plant and grown on a synthetic nutrient medium. The choice of this system was based on several considerations. First, pea seeds, like the seeds of a number of other plants, during certain stages of their development accumulate much greater amounts of gibberellin than are found in seedlings or in the organs of older plants. Our determinations were made mostly on samples of ten seeds; to obtain reasonably reliable results with pea seedlings a minimum of several hundred seedlings are needed per sample. Secondly, in most plants or plant parts the addition of retardants causes a reduction in growth. Even if a reduction in the concentration of endogenous gibberellin is found, the relations between cause and effect remain equivocal. The accumulation of large quantities of gibberellins in developing seeds